

UNIV. OF
TORONTO
LIBRARY



Med
J.

THE JOURNAL
OF
CANCER RESEARCH

CANCELLED

EDITED BY
WILLIAM H. WOGLOM

ASSOCIATE EDITOR
FREDERICK PRIME

EDITORIAL COMMITTEE
OF
THE AMERICAN ASSOCIATION FOR CANCER RESEARCH

JOSEPH C. BLOODGOOD
Johns Hopkins University

H. GIDEON WELLS
University of Chicago

LEO LOEB
Washington University

JAMES EWING
Cornell University

ERNEST E. TYZZER
Harvard University

VOLUME III

BALTIMORE, MD.

1918

155-335-
1916/20
CANCELLED

112

200

100

100

CONTENTS

NUMBER 1, JANUARY, 1918

Experimental Study of the Pathogenesis of Carcinoma. Katsusaburo Yamagiwa and Koichi Ichikawa	1
A Histological Study of Heterologous Tumor Grafts. F. D. Bullock and G. L. Rohdenburg.....	31
Significance of the Cartilage in a Carcinochondrosarcoma of the Mouse. William H. Woglom.....	47
Growth of Tumors in the Chick Embryo. Holland N. Stevenson.....	63
The Part played by the Hydroxy-Benzol Radical in the Acceleration of the Growth of Carcinomata by Cholesterol and by Tethelin. T. Brailsford Robertson and Theo. C. Burnett.....	75
Cases of Typical and Atypical Lymphosarcoma. George M. MacKenzie...	93

NUMBER 2, APRIL, 1918

Researches on the Cancerous Diseases in Norway. F. G. Gade.. . . .	107
Some Phases of Radium Action with Special Reference to the Hematopoietic System. John A. P. Millet and Theodor Mueller	127
The Influence of Heat and Radium upon Induced Immunity Against Transplanted Animal Tumors. G. L. Rohdenburg and F. D. Bullock.....	181
Fluctuations in the Growth Energy of Malignant Tumors in Man, with Especial Reference to Spontaneous Recession. G. L. Rohdenburg. . .	193

NUMBER 3, JULY, 1918

Experimental "Carcinomata" of Animals and their Relation to True Malignant Tumors. F. D. Bullock and G. L. Rohdenburg.....	227
Adenocarcinoma or Adenoma of the Liver in a Mouse. Shigemitsu Itami..	275
An Hereditary Tumor in the Fruit Fly <i>Drosophila</i> . Mary B. Stark.....	279
The Biological Treatment of Cancer. G. Fichera.....	303

NUMBER 4, OCTOBER, 1918

Occupational Cancer. H. C. Ross.....	321
Multiple Skin Metastases from Cancer of Internal Organs. Nobuyoshi Suzuki.....	357
Index	389



R. Weil

Major Richard Weil, M. R. C.

If, in the paths of the world,
Stones might have wounded thy feet,
Toil or dejection have tried
Thy spirit, of that we saw
Nothing; to us thou wert still
Cheerful, and helpful, and firm.

—*Matthew Arnold.*

The JOURNAL OF CANCER RESEARCH records with the deepest regret the loss of its Managing Editor, Dr. Richard Weil.

Hardly had war been declared before Dr. Weil, with characteristic generosity, offered himself to the Government. He shortly received a Captain's commission in the Medical Reserve Corps and was detailed to Fort Benjamin Harrison, Indiana, where all the summer was spent in arduous preparation for new duties. Even among such a large group his ability could not but be noticed, and it was no surprise to hear, in October, that he had been advanced to the rank of Major and appointed Chief of Medical Service at the Base Hospital, Camp Wheeler, Macon, Georgia. Here he commenced his responsible task, and here, on November 19, 1917, it was completed; for, having driven himself to the point of exhaustion in attending the unusually large number of patients committed to his care, he was unable to overcome an attack of pneumonia from which one who had lived less unselfishly might have been saved.

Dr. Weil was born in New York City in 1876, and graduated from Columbia College in 1896. After having received the degree of M.D. from the College of Physicians and Surgeons, Columbia University, in 1900, he entered the German Hospital, New York, to serve as interne, and, following this, spent two years in study abroad, chiefly in Marchand's laboratory. He then returned to New York to devote himself to scientific medicine.

In 1904, he became pathologist to the German Hospital, whence he published a treatise on "Urine and Feces in Diagnosis," in collaboration with Drs. Hensel and Jelliffe. Two years later he joined the staff of the Huntington Fund for Cancer Research, and at the Loomis Laboratory initiated those investigations on the reactions of cancer and immune sera which thereafter absorbed his chief interest. His first studies concerned the hemolytic and antitryptic action of cancer serum. In 1910, he showed that the blood of syphilitics is extremely sensitive to hemolysis by cobra venom and, on the basis of this reaction, devised an exceptionally delicate clinical test for luetic infection. In a series of experiments on anaphylaxis, he elucidated this obscure condition to a notable degree by demonstrating that the sensitization is essentially cellular in origin.

In 1913, Dr. Weil became assistant director of cancer research and attending physician at the Memorial Hospital and gave much of his time to developing the organization of that institution. Here he perfected and employed the method of transfusing citrated blood. Having served also on the staffs of the Montefiore Home and Mt. Sinai Hospital, he had acquired an unusually high degree of diagnostic skill in a wide field of medicine, and, in 1915, was made professor of experimental medicine in Cornell University.

His extensive horizon is amply evident from the large number of societies to which he belonged; these included the New York Academy of Medicine, the American Medical Association, the New York State and County Medical Societies, the New York Pathological Society, the Association of American Physicians, the American Association of Pathologists and Bacteriologists, the American Association of Immunologists, and the American Society for the Control of Cancer.

His chief interest, however, was always reserved for the problems of cancer. Thus, he became one of the founders of the American Association for Cancer Research and, at the request of its Council, assumed the burden of establishing this JOURNAL, which he served most faithfully and, by reason of his thorough knowledge of the literature of cancer, most effectively. By his

untimely death American cancer research loses one of its most scholarly and accomplished workers.

The following is a list of Dr. Weil's later and more important publications:

Hemolytic properties of organ and tumor extracts. *Journal of Medical Research*, 1907, xvi, 287.

The hemolytic reactions of the blood in dogs affected with transplantable lymphosarcoma. *Archives of Internal Medicine*, 1908, i, 23.

The hemolytic reactions in cases of human cancer. *Journal of Medical Research*, 1908, xix, 281.

Avoidance of hemolysis in transfusion. *American Journal of Surgery*, 1909, xxiii, 96.

Resistance of human erythrocytes to cobra venom. *Journal of Infectious Diseases*, 1909, vi, 688.

An experimental study of the antitryptic activity of human serum. *Archives of Internal Medicine*, 1910, v, 109.

The antitryptic activity of human blood serum; its significance and its diagnostic value. *American Journal of Medical Sciences*, 1910, cxxxix, 714.

The biochemical investigation of malignant tumors and its diagnostic applications. *Journal of the American Medical Association*, 1910, lv, 1532.

Properties of ascitic fluids, especially in cases of cancer. *Journal of Medical Research*, 1910, xxiii, 85.

The nature of anaphylaxis, and the relations between anaphylaxis and immunity. *Journal of Medical Research*, 1913, xxvii, 497.

A study of the blood in rats recovered from implanted sarcoma. *Journal of Experimental Medicine*, 1913, xviii, 390.

Studies in anaphylaxis:

I. On the sensitizing dose in active anaphylaxis.

II. On passive sensitization by heterologous immune serum. Its duration and its prevention.

III. The phenomena of so-called saturation and displacement in anaphylaxis.

IV. "Saturation" and "displacement" in active sensitization, and in passive homologous sensitization. *Journal of Medical Research*, 1913, xxviii, 243.

The intravascular implantation of rat tumors. *Journal of Medical Research*, 1913, xxviii, 497.

The effects of colloidal copper, with an analysis of the therapeutic criteria in human cancer. *Journal of the American Medical Association*, 1913, lxi, 1034.

Studies in anaphylaxis:

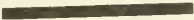
V. Desensitization: its theoretical and practical significance. *Journal of Medical Research*, 1913, xxix, 233.

VI. A study of the cellular theory by the graphic method.

Studies in Anaphylaxis:

- VII. The relation between antibody content and lethal dose in anaphylaxis.
- VIII. The function of circulating antibody and the avidity of cellular antibody.
- IX. The relation between partial desensitization and the minimal lethal dose in anaphylaxis.
- X. The persistence of intracellular antigen as a factor in immunity.
- XI. The share of intracellular antigen in immunity and in desensitization. Theoretical considerations. *Journal of Medical Research*, 1914, xxx, 87, 299.
- XIII. Activation of antibody by the cell. *Journal of Medical Research*, 1915, xxxii, 107.
- Sodium citrate in the transfusion of blood. *Journal of the American Medical Association*, 1915, lxiv, 425.
- Chemotherapy and tumors. *Journal of the American Medical Association*, 1915, lxiv, 1283.
- The autolysin treatment for cancer. *Journal of the American Medical Association*, 1915, lxv, 1641.
- The treatment of parotid tumors by radium. *Journal of the American Medical Association*, 1915, lxv, 2138.
- Studies in anaphylaxis:
 - XIV. On the relation between precipitin and sensitizin.
 - XV. Equilibrium in precipitation reactions. Equilibrium in combination.
 - XVI. Dissociation.
 - XVII. On the coexistence of antigen and antibody in the body. *Journal of Immunology*, 1916, i, 1.
- Immunological studies in pneumonia. *Journal of Experimental Medicine*, 1916, xxiii, 1.
- Note on skin reaction in pneumonia. *Journal of Experimental Medicine*, 1916, xxiii, 11.
- Chemotherapeutic experiments on rat tumors. *Journal of Cancer Research*, 1916, i, 95.
- Studies in anaphylaxis:
 - XVIII. The mechanism of delayed shock. *Journal of Immunology*, 1916, ii, 95.
 - XIX. Simultaneous injections of antigen and antiserum. *Journal of Immunology*, 1916, ii, 109.
- The immune reaction to tuberculous infection. *Journal of the American Medical Association*, 1917, lxxiii, 972.
- The vasomotor depression in canine anaphylaxis. *Journal of Immunology*, 1917, ii, 429.
- The relation between antigen and antibody in the living animal. *Journal of Immunology*, 1917, ii, 399.
- Studies in anaphylaxis:
 - XX. The reciprocal relations of antigen and antibody within the cell. *Journal of Immunology*, 1917, ii, 469.

Studies in anaphylaxis:

- XXI. Anaphylaxis in dogs. A study of the liver in shock and in peptone poisoning. *Journal of Immunology*, 1917, ii, 525.
 - XXII. Anaphylactic reactions of the isolated dog's liver. *Journal of Immunology*, 1917, ii, 571.
- 

EXPERIMENTAL STUDY OF THE PATHOGENESIS OF CARCINOMA¹

KATSUSABURO YAMAGIWA AND KOICHI ICHIKAWA

*From the Pathological Institute of the Medical College of the Imperial University,
Tokyo, Japan*

Received for publication, May 24, 1917

The problem of the etiology of tumors, and in particular of carcinoma, has been investigated for a great many years, but has not yet been solved. Many suggestions have been offered, among which Virchow's and Cohnheim's are especially prominent; both of these, however, were the result of clinical experience rather than of experimental study; hence, the adherents of these hypotheses have tried subsequently to establish them by means of experiments, the object of which has been artificially to produce carcinoma. But all attempts have proved negative, until Fibiger (1), of Copenhagen, finally succeeded in producing papilloma and carcinoma in the stomach and the esophagus of rats by infecting them with spiroptera; the origin of these new growths he referred to irritation of the mucous membrane of the stomach and the esophagus by the parasite. Thus the soundness of Virchow's irritation hypothesis has been demonstrated experimentally for the first time.

To this problem we also have devoted ourselves for four years (12, 13, 14, 15, 16, 17, 18, 19, 20), pursuing experiments in accordance with Yamagiwa's view (2), which he has expressed as follows: The repetition or continuation of chronic irritation may cause a precancerous alteration in epithelium previously normal. If the irritant continue its action, carcinoma may be the outcome, even though no specific agent has been interpolated.

¹ The authors have not read the proof of this article.

For our experiments, we have employed mainly the ear of the domestic rabbit, an organ in which no spontaneous new growth has ever been reported. Among the methods of mechanical or chemical irritation chosen, the painting of coal-tar upon the inner surface of the ear has been the most efficacious in producing carcinoma.

The investigations are being continued, but we wish to report here a summary of the results up to June 30, 1916.

We have tried various methods (table 1) of irritating the epithelium and subcutis, and with each of them have produced, as Fischer (9), Haga (10), Jores (3) and others have done, an

TABLE 1
Methods, and number of experiments

	EXPERIMENT				TOTAL
	I	II	III	IV	
	Coal-tar painting of inner surface of ear every two or three days	Coal-tar painting of outer surface of ear every two or three days	Coal-tar painting of incised wounds on margin of ear every two or three days	Coal-tar painting on outer surface of ear every two or three days after repeated intradermal injection of scarlet oil	
Number of rabbit ears employed.....	50	26	31	30	137

atypical growth of the epithelium (12); but of all the methods, painting with coal-tar was most effective, as has been said, and it had the additional advantage, furthermore, of being a simple procedure.

It was hoped that epithelium which had once begun a career of atypical growth would proliferate more and more atypically as the applications of coal-tar were continued, until at last it acquired a true malignant character; for the occurrence of carcinoma in coal-tar workers has become well known since the investigations of Vollkmann (4), Liebe (4), Tillmanns (5), and Schuchardt (6); and while coal-tar carcinoma has not yet been experimentally produced, it is nevertheless conceivable that one

might succeed if suitable methods of applying this irritant could be discovered.

Only Cazin (7) and Bayon (8) appear to have employed coal-tar for an experimental investigation of the pathogenesis of carcinoma. The former brushed this material upon the inner surface of a dog's ear, while the latter injected hypodermically a mixture of watery extract of coal-tar and lanolin; neither, however, produced more than a temporary atypical growth of the epithelium.

TABLE 2

A. The result with regard to the rabbits in four earlier experiments which have survived over 70 days

NUMBER OF RABBITS AND EARS IN EACH EXPERIMENT		NUMBER OF EARS BEARING FOLLICULO-EPITHELIOMA AMONG THE SURVIVING RABBITS	PERCENTAGE RESULT BETWEEN 2 AND 3
Rabbits	Ears		
15	23	20	87
6	6	4	67
7	13	7	54
10	10	1	10

B. The result with regard to rabbits which have survived over 150 days

9	13	13	100
3	3	3	100
4	7	7	100
6	6	1	17

The results of our own investigations with coal-tar have gradually approached our expectations; *folliculoepitheliomata*² have arisen in nearly all ears after a period longer than 100 days (table 2) from the beginning of the application, numbering in one instance twenty on a single ear. The size of the lesions varied from that of a grain of rice to that of a sparrow's egg. Some later showed a more malignant character and it could be proved, in fact, both macroscopically and microscopically, that we had produced from them eight cases of carcinoma in its

² We employ the term *folliculoepithelioma* for papillomatous new growths which reach the size of a rice grain; a full explanation of this term will be given in a succeeding paragraph.

earliest stage, sixteen cases of carcinoma in an early stage, and seven cases of still more fully developed carcinoma. Furthermore, we were able to demonstrate the presence of metastases in the lymph nodes at the root of the ear and in the submaxillary region in two cases among the last seven of carcinoma.

CLINICAL COURSE OF THE LESIONS

We have divided the course of events into four periods: a first period, characterized by atypical growth of the epithelium (before the appearance of folliculoepithelioma); a second, marked by the appearance of folliculoepithelioma benignum; a third, in which folliculoepithelioma malignum (carcinomatous alteration in a folliculoepithelioma) arises; and a fourth period, that of metastasis.

First period: Atypical growth of the epithelium. After application of coal-tar had been begun the rabbits appeared uneasy and the irritated ear soon became swollen. One week later the epithelium, especially that of the hair follicles, appeared hyperkeratotic; cystic dilatation of the follicles set in, and keratin was retained in them. At the same time, also, the hair began to come out. These changes became gradually more distinct during the period of 30 to 360 days.

Second period: Appearance of folliculoepithelioma. About 50 days after irritation had been begun, we noticed certain circumscribed regions in which the epithelium of one or more hair follicles was hyperkeratotic to a high degree. These areas became gradually more and more elevated, and eventually developed into papillomatous new growths which were very variable in size and shape, though roughly divisible into two groups: *Pedunculated* and *sessile*. The largest of the former attained approximately the size of a nut, some, however, being larger. In some cases, the interstitial connective tissue increased in amount, the growths then appearing like fibroepitheliomata or fibromata. The sessile tumors measured over 1 cm. in diameter across the base. On the cut surface of all these growths there were visible a number of dilated hair follicles, in

which keratin had accumulated. Many of the tumors continued to grow after the irritant had been withdrawn (provided it had been applied for a sufficiently long period), more and more of the horny substance accumulated in the dilated follicles, and finally these neoplasms came to resemble the cutaneous horns found in the human subject. If broken off at the base, new horns grew again at the same site or from the neighbouring epithelium.

Third period: Production of carcinoma. In the course of continued application of coal-tar to these new growths, the cut surface of some of them became so irregular that we could not differentiate macroscopically one hair follicle from another. The microscope showed a high degree of atypical proliferation of the epithelium (carcinoma in its earliest stage). The surface of these new growths then ulcerated, the tumors increased in size, and the walls of the ulcers gradually thickened, so that they came to resemble rodent ulcer. Microscopic examination disclosed an early stage of carcinoma. Certain of these growths continued to increase in dimension until they imitated even macroscopically the carcinomata of man. Thus our first and fifth cases presented elevated nodules which had, under the microscope, the appearance of a carcinoma more advanced than those described as being in an early stage. The second, fourth, sixth, and seventh cases of carcinoma arose from sessile folliculoepitheliomata, while the third developed from a pedunculated tumor. The second, third, fourth, sixth, and seventh cases of carcinoma were, both macro-and microscopically, the most typical carcinomata in our series.

Fourth period: Metastasis. The third, fourth, sixth, and seventh cases, among these seven examples of experimentally produced carcinoma, exhibited a swelling of the regional lymph nodes, and in the fourth and the sixth cases these nodes were proved by the microscope to contain metastatic deposits from the primary tumor. The nodes of the third case were the seat of a suppurative adenitis, yet it is conceivable that they may have contained carcinomatous metastases before the infection developed; those of the seventh have not yet been extirpated, but it may be supposed that they contain metastatic deposits,

inasmuch as the primary tumor was more advanced than either of the two in which metastasis was definitely proved.

Microscopical observation demonstrated that the carcinomata originated in folliculoepitheliomata, passing step by step through various stages until they reached that of completely developed carcinoma. Since the sequence of events was similar in all, we shall briefly describe here only the representative cases of carcinoma (third, fourth, sixth, and seventh cases).

Third case of carcinoma

This growth arose on the inner surface of the right ear in a black female rabbit, about two years old, in which applications of coal-tar had been begun on December 15, 1914, and repeated every two or three days. Seventy-five days after the first painting, a pedunculated papilloma was found in the irritated area; this tumor grew slowly until, 111 days later, it had become more and more hyperkeratotic, had assumed the sessile form, and had ulcerated. It spread along the surface and its edges thickened gradually, so that it came to resemble a rodent ulcer; but it seemed to invade the deeper tissues also, an area on the outer surface of the ear opposite to it becoming gradually elevated and finally ulcerating. After 130 days, the ear was perforated by an opening that soon became large enough to admit the little finger, and finally extended to the free border (fig. 12).

On the 151st day small pieces from the thickened wall of this perforating ulcer were removed for microscopical examination. The microscope showed that the new growth had already become malignant, resembling a carcinoma in its epithelial pearls and columns of epithelium; furthermore, there could be demonstrated the formation of thrombi in the veins, and a remarkable infiltration of the surrounding tissues.

The lymph nodes at the root of the affected ear began to swell after perforation had occurred, and reached the size of a large nut before the animal died. As they were the seat of suppurative adenitis, it is impossible to say whether or not they contained metastases.

The tumor in this rabbit continued to increase in size until the animal died from emaciation on the 180th day.

Fourth case of carcinoma

This is a case in which the occurrence of metastasis was proved.

The new growth arose on the inner surface of the ear of a two year old, black, male rabbit, weighing 2560 grams at the beginning of the experiment (June 16, 1915). One hundred and thirty-three days later we observed that a small papillomatous growth (the early phase of folliculoepithelioma) the size of a grain of rice, had arisen from a whitish spot on the irritated surface. On the 165th day its diameter measured 2.3 cm. and its surface had become ulcerated. The ulcerated surface was elevated above the level of the surrounding epithelium, and the wall of the ulcer gradually thickened, so that the growth came to resemble macroscopically a rodent ulcer. Two-thirds of the tumor was removed, together with the ear cartilage and the epithelium of the opposite side (outer surface), and the growth was inoculated into the subcutaneous tissues of the back and the ear in 20 rabbits. Microscopical investigation of the extirpated sample proved that the neoplasm had already assumed the characteristics of carcinoma, the nodule exhibiting those features of "carcinoma in its early stage" which we shall describe in a following paragraph.

The remainder of the tumor, left in situ, showed an inclination to diminish in size for a while after the operation, but it gradually regained its dimensions and on the 273d day (76 days after the operation, March 15, 1916) its diameter had reached 2.8 cm. At that time we observed a nodule on the outer surface of the ear in a situation corresponding to that of this growth on the inner surface; the new nodule, like the primary tumor, finally ulcerated. The irritant was then discontinued to see whether the tumors would continue to grow in its absence. This proved to be the case, for the nodule grew slowly, spreading along the surface, and the lymph nodes at the root of the ear began to swell; furthermore, the animal commenced

to lose weight. On the 294th day both ulcers had increased still more in size, the regional lymph nodes had enlarged to about the size of a peanut, and emaciation had become even more noticeable.

On the 350th day the primary ulcer on the inner surface of the ear measured 5.2 cm. by 5.3 cm., and the secondary one on the outer surface 3.5 cm. by 4.5 cm. One of the lymph nodes at the root of the ear had attained nearly the size of a sparrow's egg, and the others the size of a peanut. On the 351st day these nodes were extirpated, and inoculated, in small fragments, into the subcutaneous tissue of the back in 10 rabbits. That portion reserved for histological examination contained no metastatic deposits, and the result of the transplantation was negative. On the 366th day (June 16, 1916) the animal died, extremely emaciated, its weight having been reduced almost to half the body weight at the time of the first coal-tar application. The tumor had continued to grow, and when the rabbit died the neoplasm was about twice as large as when the irritant was discontinued (on the 273d day); its size was now 5.5 cm. by 6.5 cm., while the secondary nodule was 4.5 cm. by 5.2 cm.

At the post mortem examination, solid and cystic metastatic nodules varying from miliary size to the dimensions of a soja peanut were discovered in the two lymph nodes at the root of the ear and in one of the submaxillary nodes; the former were found, upon microscopic examination, to consist of accumulations of cancer cells, while the latter contained a mucous fluid in which yellowish flocculi, composed of degenerated epithelial cells and leucocytes, were suspended. The walls of such cysts were covered with a many-layered epithelium which, in several places, had formed epithelial pearls and was infiltrating the lymph sinus and the surrounding tissues. One of the enlarged submaxillary nodes yielded similar findings. In no other organ, however, were we able to find either gross or microscopical evidence of metastasis.

Sixth case of carcinoma

In this case, also, the occurrence of metastasis was proved.

The tumor arose on the inner surface of the right ear of a white female rabbit about two years old, and weighing 2750 grams when the experiment was begun on July 2, 1915.

On the 261st day we observed a small elevation on the irritated surface; on the 292d day this nodule, which now measured 0.7 cm. by 0.5 cm., ulcerated. On the 302d day its diameter was 1 cm., and on the 312th day two lymph nodes at the root of the ear began to enlarge, the tumor having, in the meantime, attained a diameter of 1.3 cm.

On the 322d day (May 20, 1916) the animal began to emaciate; on this date the coal-tar was discontinued. On the 332d day the two swollen lymph nodes had reached about the size of a peanut, and the ulcerated tumor continued to enlarge, even though the applications of coal-tar had been stopped. On the 337th day the new growth measured 1.0 cm. by 1.5 cm., and the enlarged nodes had attained almost the size of a nut. The lymph nodes were extirpated and small fragments inoculated subcutaneously into 10 rabbits, but the result was negative. At the operation these nodes were found adherent to the surrounding tissues, a condition which the microscope proved to be due to the infiltrative growth of carcinomatous metastases into them. The cut surface of the node exhibited a cystic nodule about the size of a pea, with contents similar to those of the one just described. The cyst was lined by many layers of cancer cells and other parts of the node contained accumulations of these elements, but epithelial pearls could not be demonstrated. The node, therefore, undoubtedly contained metastases.

On the 338th day the rabbit died, extremely emaciated (1750 grams). The ulcerated folliculoepithelioma resembled human carcinoma in its histological aspects. The rest of the post-mortem findings were negative; in particular, neither macroscopic nor microscopic evidence of metastases in remote regions could be discovered.

Seventh case of carcinoma

This tumor developed from a cutaneous horn. It arose on the inner surface of the right ear of a two year old, brown, female rabbit after long continued irritation with coal-tar (November 19, 1914, to June 12, 1916); after the 205th day the irritant was discontinued, in order that the behavior of the lesion in the absence of its exciting cause might be observed.

By the 205th day two sessile and two pedunculated folliculoepitheliomata had arisen, and on the 305th day many new sessile folliculoepitheliomata were observed; nearly all these tumors continued to enlarge and developed into cutaneous horns, one of which gave origin to a carcinoma (seventh case); it is this one in particular which we now wish to discuss.

This, the seventh case of carcinoma, arose on the 286th day from a diffusely elevated spot on the irritated surface, which elevated and enlarged gradually until it had become a sessile folliculoepithelioma, and, by the 508th day, a cutaneous horn; it grew both in length and width, measuring on the 513th day 1.5 cm. by 1 cm. It was conical in shape, the free end was hard and dry, and the base elastic and soft. On the 518th day a small area at the base ulcerated, and on the 520th day the free end of the horn fell off; the portion now remaining consisted of an ulcer with edges elevated 0.5 cm. from the surrounding epithelium and an even, finely granulated surface.

On the 543d day (May 15, 1916) the diameter of this ulcer had reached 1.5 cm., and a small diffuse elevation was noticed on the opposite (outer) surface of the ear, which became gradually larger and at last ulcerated like the primary folliculoepithelioma on the inner surface. There was considerable venous engorgement. On the 565th day the primary ulcer was 2.3 cm. by 1.5 cm., and the lymph nodes at the root of the ear began to swell, one of them being about the size of a soja peanut; both ulcer and nodes continued to increase in size. A fragment removed for microscopical examination had all the characteristics of a rodent ulcer; the epithelium had infiltrated into the lymph vessels and the veins and formed a thrombosis of car-

cinoma cells in them; furthermore, it had penetrated the ear cartilage and had reached the subcutaneous tissue on the opposite side, where it produced on the outer surface the ulcer just mentioned. This case is still under observation, the animal being still well, and the lymph nodes will be transplanted as soon as they are large enough.

The four cases of carcinoma just described exhibited the following macroscopic and microscopic signs of spontaneous carcinoma: (1) Exhaustion and emaciation, resembling the cachexia of cancer patients. The animal in which the seventh case of carcinoma arose is not yet emaciated, it is true, but this may be because the carcinoma is still fairly recent. (2) The tumors infiltrated the subcutaneous tissue, the lymph channels, and the blood-vessels, and exhibited the histological features characteristic of rodent ulcer. Again, these carcinomata grew not only in the presence of the irritant, but also after it had been discontinued; thus in the fourth case of carcinoma the tumor doubled in size in the 93 days following the withdrawal of the coal-tar applications. (3) The presence of metastases was proved in the regional lymph nodes in the fourth and sixth cases.

Such facts make it evident that these experimentally produced carcinomata closely resemble carcinoma in man, and especially that type found among workers in coal-tar.

HISTOLOGY OF THE LESIONS

As we have in the preceding paragraphs, divided the course of our experiments into four periods, according to the macroscopical findings, so we wish to discuss in the following pages the microscopical appearances characteristic of these four periods.

First period: Atypical growth of the epithelium. The epithelium, and especially that at the periphery of the hair follicles, gradually undergoes hyperplasia; (1) each layer increases considerably in thickness; (2) many symmetrical mitoses are found in its basal layer; (3) the hair follicles become cystic; (4) the basal layer grows irregular in outline, owing to the projection

of processes which ramify in the surrounding subcutaneous tissues. These are the earliest stages in the development of folliculoepithelioma benignum and malignum. Furthermore, the blood-vessels, especially the veins and capillaries, soon dilate, while eosinophiles and lymphocytes escape into the neighboring connective tissues. The duration of this period is very variable; according to the individual animal it may occupy from 30 to 350 days, the average being about 100 days.

Second period: Appearance of folliculoepithelioma. The circumscribed papillomatous elevations on the irritated surface, which we have already described, consist of one or more hair follicles; in them, hyperplasia and hyperkeratosis of the epithelium are present in high degree, the process resembling that seen in the first period already mentioned, except that it is more advanced. From these elevations arose new growths, which we divide into two general types, *pedunculated folliculoepithelioma* and *sessile folliculoepithelioma*, though they are very variable both in size and shape.

(1) *Pedunculated folliculoepithelioma:* This arises when hair follicles in the central or the peripheral part of the papillomatous epithelial elevation are lifted upward by the pressure of epithelial growth, and continue to proliferate. As these new growths arise primarily from the epithelium of the hair follicle, we call them folliculoepithelioma rather than papilloma. In their subsequent growth, some of these pedunculated folliculoepitheliomata underwent an increase in their connective tissue, thus becoming fibromatous; others came to resemble the sessile type; and still others (10 in all) developed into cutaneous horns.

(2) *Sessile folliculoepithelioma.* This type arose from one or more hair follicles when the papillomatous epithelium showed a higher degree of hyperplasia and hyperkeratosis. The cystic hair follicles are arranged almost directly on the ear cartilage, and the epithelium and corium extending upward between them form septa or papillary processes, the spaces between which are filled with collections of concentric keratinized epithelial cells. Hence these new growths have wide bases, and are

elevated but little from the surface of the skin; occasionally, however, one or more hair follicles may push upward, as in the pedunculated variety. Thirty-four of these sessile folliculo-epitheliomata developed into cutaneous horns when we stopped the coal-tar.

Some of these cutaneous horns diminished gradually in size and at last disappeared, but an equal number continued to grow for 365 days after the coal-tar had been discontinued.

The blood-vessels, especially the veins and capillaries, become greatly dilated, and eosinophiles and lymphocytes are found in the interstitial connective tissue. Many symmetrical bipolar mitotic figures occur in the basal layer of the epithelium. Mucous degeneration of the interstitial tissue was not found in these folliculoepitheliomata.

Third period: Production of carcinoma. This period we have divided into three stages:—(1) earliest stage of carcinoma; (2) early stage of carcinoma; (3) fully developed carcinoma.

(1) Earliest stage of carcinoma. It is difficult to differentiate these very young carcinomata from benign folliculo-epithelioma. However, the following characteristics will help in the differential diagnosis. All or a part of the epithelium of these new growths assumes a fainter stain with hematoxylin than does normal epithelium or that of the benign folliculo-epithelioma; the sprout-like processes developed by atypical proliferation of the basal epithelium of the hair follicle become more angular at their basal layer, and the processes grow very irregular in thickness; the interstitial connective tissue becomes loose or shows a slight mucous degeneration; lateral and downward penetration of the cancerous epithelium can be demonstrated.

We believe that such changes as these must be the initial step in the transformation to carcinoma.

Eight cases of carcinoma in the earliest stage were discovered among seven irritated ears (see tables 3, 4, and 5).

(2) Early stage of carcinoma. By prolonging the irritation, we have produced many cases of folliculoepitheliomata exhibiting more advanced changes than are found in the earliest stage

of carcinoma. Such tumors show the following characteristics:—the interstitial connective tissue becomes loose and edematous, the sprout-like processes in the basal layer of the hair follicles are more angular than in the first stage, these sprouts grow even more irregular in thickness, lateral and downward invasion takes place to a higher degree than in the previous stage, and the invading processes anastomose with one another to form a network; the intercellular spaces become wider (*dis-*

TABLE 3
Experimentally produced malignant folliculoepithelioma

CASES		EXPERIMENTAL SERIES	SEX	SIDE	DAYS ELAPSED BEFORE MICRO- SCOPICAL DIAGNOSIS COULD BE MADE	DAYS TO DEATH OF RABBIT
Carcinoma.....	1st case	III	♀	Right	179	222
	2d case	I	♀	Right	103	103*
	3d case	I	♀	Right	151	180
Carcinoma in its early stage.....	1st case	I	♀	Right	103	103*
	2d case	I	♀	Left	103	103*
	3d case	I	♀	Right	103	103*
	4th case	I	♂	Left	103	183
Carcinoma in its earliest stage.....	1st case	I	♀	Left	103	103*
	2d case	II	♀	Left	156	156
	3d case	II	♀	Left	194	255

* The second case of complete carcinoma, the first, second, and third cases of carcinoma in its early stage, and the first case of carcinoma in its earliest stage, were all seen in one rabbit.

sociation) and individual cells leave the main group (*emancipation*); the epithelium takes a fainter stain with hematoxylin than does normal epithelium or that exhibiting atypical growth; two among these cases showed invasion of the veins, and one into the lymphatic channels; the dilatation of the capillaries and the veins is generally more advanced than in the first period.

These are, in general, the characteristics of our carcinomata in their early stage, but the process varies in intensity, and some of the lesions resembled complete carcinoma. The find-

TABLE 4

CASES		SEX	SIDE	DAYS NECESSARY FOR MICROSCOPIC DIAGNOSIS	DAYS TO DEATH OF RABBIT	REMARKS
Carcinoma ..	4th case	♂	Right	197	366	Metastasis proved in the regional lymph nodes
	5th case	♀	Right	120	120	This rabbit bore the eleventh case of carcinoma in its early stage
	6th case	♀	Right	338	338	Metastasis proved in the regional lymph nodes; this rabbit bore three cases of carcinoma in its early stage
Carcinoma in its early stage.....	5th case	♀	Left	338	338	These three cases of carcinoma in its early stage were produced in the same rabbit which bore the sixth case of carcinoma
	6th case	♀	Right	338	338	
	7th case	♀	Right	338	338	
	8th case	♂	Left	238	238	This rabbit bore the fourth case of carcinoma in its earliest stage
	9th case	♀	Right	128	128	
	10th case	♂	Right	118	118	
	11th case	♀	Left	120	120	This rabbit bore the fifth case of carcinoma
	12th case	♀	Right	90	90	These cases produced in the same rabbit
	13th case	♀	Left	90	90	
	14th case	♀	Left	90	90	
Carcinoma in its earliest stage.....	15th case	♂	Right	55	55	These cases produced in the same animal
	4th case	♂	Left	118	118	
	5th case	♂	Right	257	257	
	6th case	♀	Left	192	192	These cases produced in the same animal
	7th case	♀	Right	256	256	
	8th case	♀	Right	256	256	

ings are like those described by Ribbert and others in early carcinoma.

So far we have produced sixteen cases of carcinoma in its early stage among thirteen ears in ten rabbits (see tables 3, 4, and 5).

(3) Fully developed carcinoma. Our seven cases of carci-

noma, which arose after further repetition of the coal-tar applications, resembled closely in their histological characteristics the spontaneous carcinomata of man. The features distinguishing the previous stage become more advanced, especially the infiltrative growth of the epithelium. This is now really striking, the carcinomatous cells invading the surrounding subcutaneous tissue both downward and laterally, growing into the veins and the lymphatic channels, penetrating the aural cartilage, and forming ulcerated new growths of a similar nature on the opposite surface of the ear. Mucous degeneration of the interstitial connective tissue was noticed in almost all cases of car-

TABLE 5
Carcinoma developing from cutaneous horns

CASE	SEX	SIDE	DAYS NECESSARY FOR MICROSCOPIC DIAGNOSIS	DAYS TO DEATH OF RABBIT	REMARKS
7th case of carcinoma	♀	Right	565	589 (still living)	These tumors developed from cutaneous horns in the second case of cutaneous horns in the first experiment (see table 6)
16th case of carcinoma in its early stage	♀	Left	565	589 (still living)	

cinoma, especially in the second, fourth, and sixth cases. The first, second, and fifth were less developed than the other four (the third, fourth sixth, and seventh), which showed in higher degree almost all the characteristics mentioned above, and resembled spontaneous carcinoma both macroscopically and microscopically.

These seven cases of carcinoma occurred on the inner surface of seven ears in seven rabbits.

Fourth period: Metastasis. This has been already discussed under other headings.

Other problems concerning the histogenesis of experimentally produced carcinoma in the rabbit have been already suggested in previous reports, but will be taken up again in full (20).

APPENDIX

Experiments on the artificial production of cutaneous horns

Three rabbits remaining from the first experiment were used for this investigation, the purpose of which was to observe the fate of the experimentally produced new growths after painting with coal-tar had been discontinued. The experiment began on June 12, 1915. To our surprise, the great majority of the

TABLE 6
First experiment

CASES	SIDE	DAYS TREATED	DAYS AFTER TREATMENT WAS DISCONTINUED	DAYS FROM BEGINNING OF EXPERIMENT	NUMBER OF CUTANEOUS HORNS DEVELOPING FROM FOLLICULOEPITHELIOMATA			NUMBER OF FOLLICULOEPITHELIOMATA UNCHANGED OR DECREASING IN SIZE		
					From sessile folliculoepitheliomata	From pedunculated folliculoepitheliomata	Total	Sessile folliculoepitheliomata	Pedunculated folliculoepitheliomata	Total
1st.....	Left	172	384	556	8	1	9	0	0	0
	Right	172	384	556	5	0	5	0	0	0
2d.....	Left	205	384	589	1	1	2	0	1	1
	Right	205	384	589	2	0	2	0	2	2
3d.....	Left	422	135	557	3	3	6	1	0	1
	Right	422	135	557	5	5	10	1	2	3
Total.....					24	10	34	2	5	7

folliculoepitheliomata developed into cutaneous horns (see table 6). This outcome was so interesting that one of us (Ichikawa) continued this investigation into the pathogenesis of cutaneous horns (see table 7), and found that 34 among 45 sessile folliculoepitheliomata developed into horns (75%) and 10 among 19 pedunculated folliculoepitheliomata (53%). The folliculoepitheliomata which did not develop into horns gradually decreased in size and finally vanished, though a few of them first underwent a secondary fibromatous alteration.

Most of the cutaneous horns increased in size after the appli-

cations of tar had been discontinued, and, furthermore, we observed horns developing from the neighbouring epithelium. Thus there was required for the production of cutaneous horns the application of coal-tar for a certain length of time, followed by a period of freedom from irritation. In the further course of the experiment, one of these horns gradually took on the char-

TABLE 7
Second experiment

CASES	SIDE	DAYS TREATED	DAYS AFTER TREATMENT WAS DISCONTINUED	DAYS FROM BEGINNING OF EXPERIMENT	NUMBER OF CUTANEOUS HORNS DEVELOPING FROM FOLLICULOEPITHELIOMATA			NUMBER OF FOLLICULOEPITHELIOMATA REMAINING UNCHANGED OR DECREASING IN SIZE		
					From sessile folliculoepitheliomata	From pedunculated folliculoepitheliomata	Total	Sessile folliculoepitheliomata	Pedunculated folliculoepitheliomata	Total
1st.....	Left	239	141	380	2	0	2	0	0	0
2d.....	Right	239	141	380	0	0	0	1	0	1
3d.....	Left	191	147	338	0	0	0	0	1	1
4th.....	Right	191	147	338	0	0	0	1	1	2
5th.....	Left	239	141	380	1	0	1	0	0	0
6th.....	Left	223	34	257	2	0	2	0	0	0
7th.....	Right	239	18	257	0	0	0	3	0	3
8th.....	Left	239	141	380	2	0	2	0	0	0
9th.....	Left	208	48	256	1	0	1	0	1	1
10th.....	Right	208	48	256	1	0	1	0	0	0
11th.....	Left	184	54	238	0	0	0	2	1	3
12th.....	Left	264	97	361	1	0	1	2	0	2
Total.....					10	0	10	9	4	13

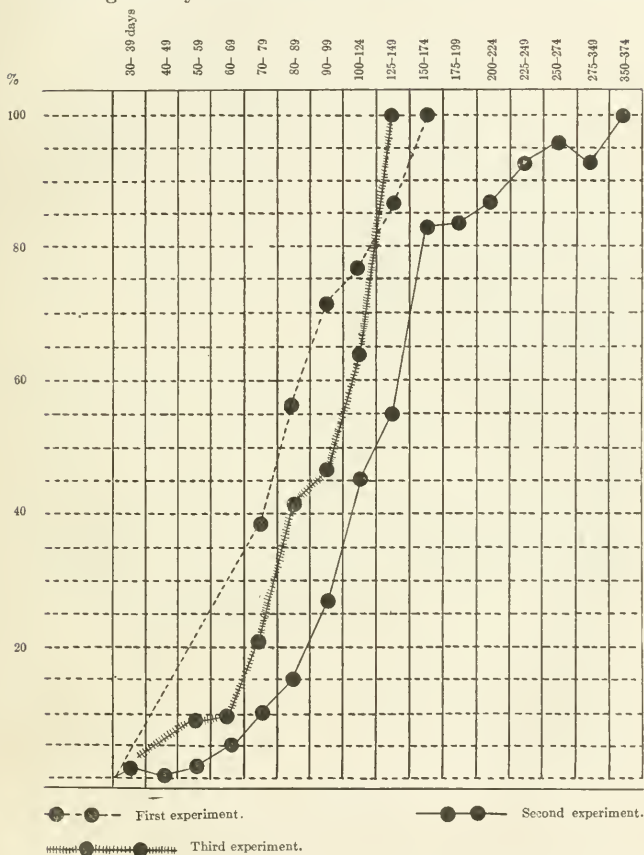
acter of an early carcinoma and another that of a carcinoma in a somewhat more advanced stage (see table 5). The details have already been discussed in the preceding paragraphs.

SUMMARY

The following conclusions may be drawn:

1. Papillomatous new growths (which we term folliculoepitheliomata) may be produced on the rabbit's ear by the ap-

plication of coal-tar for 30 to 100 days (text figure 1). The proportion of folliculoepitheliomata to the total number of ears treated gradually rises



Text Fig. 1. Gradual rise in percentage of folliculoepitheliomata with the passage of time.

2. By the repeated application of coal-tar, eight cases of carcinoma in its earliest stage, sixteen in an early stage, and seven complete carcinomata were produced. The carcinomatous change was discovered between the 55th and the 360th day; in most of the cases it was found after the 150th day.

3. The hyperkeratotic pedunculated or sessile folliculoepitheliomata produced by irritation with coal-tar continued to grow after the irritant had been discontinued, and eventually developed into cutaneous horns. Some of these horns grew for a year after the withdrawal of the coal-tar, while others fell off spontaneously; in the great majority of the latter animals new cutaneous horns grew again from the same base, or from the neighbouring epithelium, as is the case in man.

4. The seventh and sixteenth cases of carcinoma in its early stage developed from cutaneous horns about 300 days after the tar had been discontinued.

5. The presence of metastasis was microscopically proved in the regional lymph nodes in the fourth and sixth cases of carcinoma.

6. The animals which bore folliculoepitheliomata did not begin to emaciate while the new growth maintained its benign character.

7. Yamagiwa's hypothesis (2) has been confirmed: The repetition or continuation of chronic irritation may cause a precancerous alteration in epithelium previously normal. If the irritant continue its action, carcinoma may be the outcome, even though no specific agent has been interpolated.

In conclusion, we desire to acknowledge our indebtedness to the Japanese Cancer Research Fund for its support in this work, to Dr. Mann and Dr. Horiuchi for their courtesy in correcting our English, and to Dr. Kikuta for the photographs and photomicrographs.

REFERENCES

- (1) FIBIGER, J.: *Ztschr. f. Krebsforsch.*, 1913, xiii, 217.
- (2) YAMAGIWA, K.: *Zur Histo- u. Pathogenese des Magencarcinoms*, Tokyo, 1905; *Nisshin-Igaku*, Vol. iii, No. 4.

- (3) JORES: München. med. Wehnschr., 1907, liv, 879.
- (4) VOLLMANN, R. v.: Berl. klin. Wehnschr., 1874, xi, 218.
LIEBE, GEORG: Schmidt's Jahrb., 1892, cexxxvi, 65-76. Beitr. z. klin. Chir., 1875.
- (5) TILLMANN: Deutsch. Ztschr. f. Chir., 1880, xiii, 519.
- (6) SCHUCHARDT: Samml. klin. Vortr. (Volkman's), 1885, No. 257 (Chir. No. 80, 2195-2238).
- (7) CAZIN: Des origines et des modes de transmission du cancer, Paris, 1894.
- (8) BAYON: Lancet, 1912, ii, 1579.
- (9) FISCHER, B.: Centralbl. f. allg. Path. u. path. Anat., 1906, xvii, 868.
- (10) HAGA: Ztschr. f. Krebsforsch., 1913, xii, 525.
- (11) FIBIGER, J.: Ztschr. f. Krebsforsch., 1914, xiv, 295.
- (12) YAMAGIWA, K., AND ICHIKAWA, K.: Verhandl. d. japanischen path. Gesellschaft., Tokyo, 1914.
- (13) YAMAGIWA, K., AND ICHIKAWA, K.: Verhandl. d. japanischen path. Gesellschaft., Tokyo, 1915.
- (14) YAMAGIWA, K., AND ICHIKAWA, K.: Nihon-Iji-Shiuhō (Jap. Med. Weekly), October, 1915.
- (15) YAMAGIWA, K., AND ICHIKAWA, K.: Jour. Med. College of Imperial University, Tokyo, 1915, xv, No. 2. Jour. Med. Assn., Tokyo, 1916, xxx, No. 1.
- (16) YAMAGIWA, K., AND ICHIKAWA, K.: Verhandl. d. japanischen path. Gesellschaft., Tokyo, 1916, iv, 169.
- (17) YAMAGIWA, K., AND ICHIKAWA, K.: Nihon-Iji-Shiuhō, 1916, No. 1109.
- (18) ICHIKAWA, K.: Verhandl. d. japanischen path. Gesellschaft., Tokyo, 1916, iv, 159.
- (19) ICHIKAWA, K.: Nihon-Iji-Shiuhō, 1916, No. 1110.
- (20) YAMAGIWA, K., AND ICHIKAWA, K.: Jour. Med. College, Imperial University, Tokyo, 1917, xvii, No. 1.
- (21) BORST: Verhandl. d. deutsch. path. Gesellschaft., 1904, vii, 110.
- (22) BORST: Die Lehre von den Geschwülsten, Wiesbaden, 1902.
- (23) BORST: Würzb. Abh. a. d. Gesamtgeb. d. prakt. Med., 1906, vi, 221-268.
- (24) BORST: Ziegler's Beiträge, 1910, xlix, 639.
- (25) RIBBERT: Geschwulstlehre, Bonn, 1904.
- (26) RIBBERT: Karzinom des Menschen, Bonn, 1911.

EXPLANATION OF ABBREVIATIONS

<i>Ab.c.</i> , Cystic abscess	<i>M.c.w.</i> , Metastatic carcinoma cell layer on wall of cystic abscess
<i>C.d.</i> , Carcinomatous change in a folliculoepithelioma	<i>M.i.g.</i> , Metastatic carcinoma cells exhibiting infiltrative growth
<i>Ch.</i> , Cartilaginous layer of rabbit's ear	<i>M.l.</i> , Metastasis of carcinoma cells into lymph sinus in a lymph node
<i>D.ep.</i> , Dissociated epithelial cells	<i>N.ep.</i> , Relatively normal epithelium
<i>E.n.</i> , Network of carcinomatous epithelium	<i>P.h.</i> , Perforation
<i>F.</i> , Fibromatous portion	<i>Th.l.</i> , Thrombosis of lymph vessels caused by infiltrative growth of carcinomatous epithelium
<i>F.c.</i> , Folliculoepithelioma carcinomatousum	<i>Th.v.</i> , Thrombosis of vein caused by infiltrative growth of carcinomatous epithelium
<i>F.n.</i> , Fibromatous nodule	<i>R.</i> , Thickened wall of rodent ulcer
<i>I.ep.</i> , Infiltrative growth by carcinomatous epithelium	
<i>L.</i> , Lymphatic follicle	
<i>M.c.a.</i> , Metastatic accumulation of carcinoma cells	

PLATE 1

FIG. 1. Multiple hyperkeratotic sessile folliculoepitheliomata on inner surface of ear on 204th day. Natural size.

FIG. 2. First case of cutaneous horn, on the 399th day after first application of coal-tar, and the 217th day after its withdrawal. Natural size.

FIG. 3. Second case of cutaneous horn, on the 422d day after first application of coal-tar, and the 217th day after its withdrawal. Natural size.

FIG. 4. Fourth case of carcinoma in its early stage; inner surface of ear, 165th day. Two-thirds natural size.

FIG. 5. Fourth case of carcinoma, on the 273d day after first application of coal-tar, and the 77th day after its withdrawal. Natural size.

FIG. 6. Secondary ulcer and central nodule on outer surface of ear; fourth case of carcinoma. This picture was taken on same day as figure 5. Natural size.

FIG. 7. Fourth case of carcinoma in its earliest stage, 118th day. Hematoxylin-eosin. Zeiss microplanar 35 mm.

FIG. 8. Fourteenth case of carcinoma in its early stage, 90th day. Hematoxylin-eosin. Zeiss microplanar 35 mm.



FIG. 1.



FIG. 2.

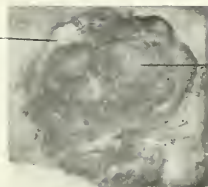


FIG. 5. (k)



FIG. 3.

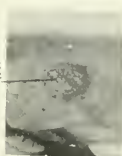


FIG. 4.

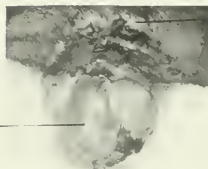


FIG. 6.

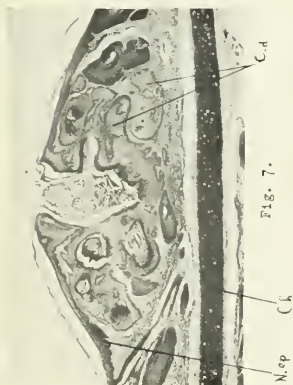


FIG. 7.



FIG. 8.

PLATE 2

FIG. 9. Thrombosis of vein by infiltrative growth of carcinomatous epithelium; thirteenth case of carcinoma in its early stage, 90th day. Hematoxylin-eosin. Zeiss projectionocular 2, obj. aa.

FIG. 10. Carcinomatous portion in fourth case of carcinoma in its early stage, 197th day. Notice dissociation of epithelial cells (D. ep.). Hematoxylin-eosin. Zeiss projectionocular 1, obj. aa.

FIG. 11. Second case of carcinoma (rodent ulcer), 103d day. Hematoxylin-eosin. Zeiss microplanar 35 mm.

FIG. 12. Third case of carcinoma, 151st day. Hematoxylin-eosin. Zeiss microplanar 50 mm.

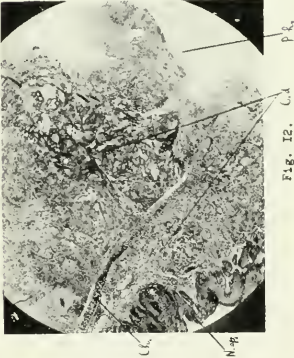
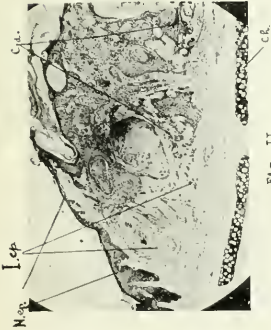
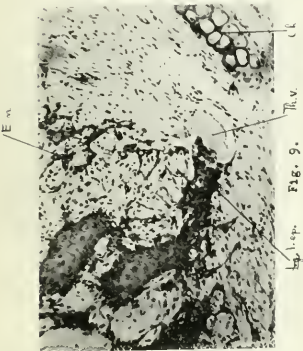


PLATE 3

FIG. 13. Fourth case of carcinoma in its early stage, 197th day. Hematoxylin-eosin. Zeiss, microplanar 50 mm.

FIG. 14. Fifth case of carcinoma, 120th day. Hematoxylin-eosin. Zeiss, projectionocular 2, obj. aa.

FIG. 15. Sixth case of carcinoma. Invasion of lymph channels at periphery of tumor. Hematoxylin-eosin. Zeiss, project. oc. 2, obj. aa.

FIG. 16. Seventh case of carcinoma, 565th day. Thrombi of cancer cells in lymph vessels. Hematoxylin-eosin. Zeiss, project. oc. 2, obj. aa.

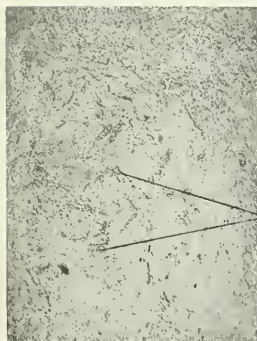


Fig. 15.



Fig. 13.

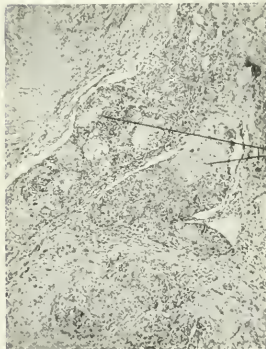


Fig. 16. Th.l.

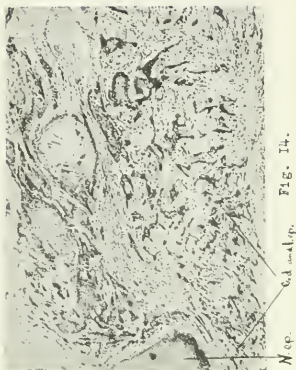


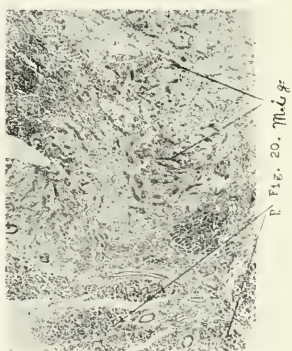
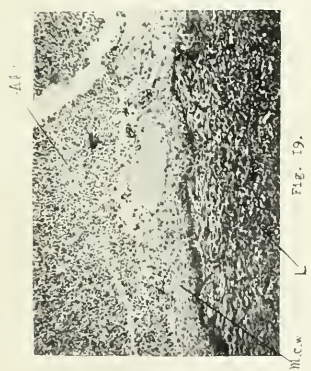
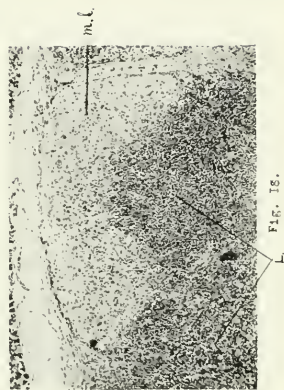
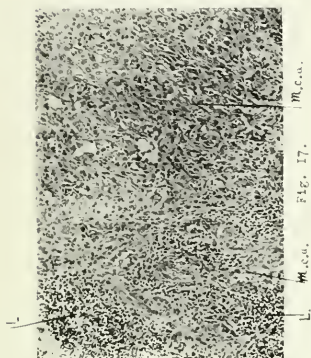
PLATE 4

FIG. 17. Metastasis in regional lymph nodes (fourth case). Hematoxylin-eosin. Zeiss project. oc. 4, obj. aa.

FIG. 18. Metastasis in regional lymph node (sixth case); cancer cells in lymph sinus. Hematoxylin-eosin. Zeiss project. oc. 2, obj. aa.

FIG. 19. Metastasis in regional lymph node (sixth case); stratified cancer cells cover the wall of a cystic abscess, which contains mucous fluid, degenerated epithelial cells, eosinophiles, and lymphocytes. Hematoxylin-eosin. Zeiss project. oc. 4, obj. aa.

FIG. 20. Metastasis in regional lymph node (fourth case). The metastatic cancer cells invaded the parotid gland, causing adhesion to the surrounding tissues. Hematoxylin-eosin. Zeiss microplanar 50 mm.



A HISTOLOGICAL STUDY OF HETEROLOGOUS TUMOR GRAFTS

F. D. BULLOCK, AND G. L. ROHDENBURG

*From Columbia University, George Crocker Special Research Fund, F. C. Wood,
Director*

Received for publication, May 31, 1917

This paper records three series of experiments upon closely related factors in tumor immunity: (a) a study of the relation between the local tissue reaction and the heteroplastic tumor graft, (b) the effect of removal of the spleen upon the growth of heterologous tumor, (c) a study of the immunizing power of one heterologous tumor upon the growth of a second heterologous tumor of different type, and the possible influence of splenectomy upon such immunity.

The presence of lymphocytes, often in large numbers, in the tissues surrounding human tumors has long been observed by pathologists, many of whom have interpreted the condition as an effort on the part of the body to combat the growth of tumor cells. Da Fano (1), the first investigator definitely to correlate the presence of lymphocytes with the immunity to transplanted tumors, explained this immunity in terms of lymphocytic activity, the plasma cell, also, being considered as a factor. He offered no explanation regarding the manner in which these cells influence the growth of the tumor graft. Da Fano's suggestion has been supported by other investigators, and especially by Murphy (2), who has noted that the presence of large numbers of lymphocytes in the region of a tumor is frequently followed by cessation of growth, and believes that the two phenomena are causally related.

In a study of homeoplastic epithelial grafts, Leo Loeb (3) has expressed the additional view that the destruction of these grafts is partly due to the action of the lymphocytes which pene-

trate them and cause the death of their cells. This action is aided by the presence of dense fibrous material which grows into the grafts, separating them into small islands, and by continued growth gradually compressing and perhaps eventually destroying the cells. It is Loeb's opinion that these two agents may act either independently of each other, or together.

A study of heteroplastic tumor grafts of several different strains was undertaken in the hope of shedding some light on the histological relationship between the tumor and its surrounding leucocytes and connective tissue, and also to determine whether this relationship remains constant regardless of the strain of tumor used for the inoculations. Although Bashford and Russell, in an investigation similar to the one recorded in this paper, have ascribed the death of the graft to the cytotoxic and cytolytic action of the body juices, it is hardly probable that they would have their conclusions regarded as otherwise than purely inferential, as there is no form of cell degeneration characteristic of an attack by cytotoxins or cytolsins. Since a histological study shows the results rather than the cause of a degeneration, only the conditions of the tissues that may lead to the death of the graft and not the ultimate causes of cell death are considered in this paper.

Two carcinomata and two sarcomata were selected for the experiment. Of the carcinomata, mouse tumor 5 shows a rapid rate of growth in the mouse, in contrast to the very slow growth of mouse tumor 58, the other carcinoma employed. Mouse sarcoma 180 and rat sarcoma 7 proliferate rapidly in the mouse and the rat respectively; the former grows progressively, the latter regresses at a comparatively early period. These tumors were inoculated into the subcutaneous tissues by needle, in the usual dose of 0.003 gram.¹ From ten to fourteen days after inoculation, the grafts and their surrounding tissues were excised and submitted to microscopical examination (serial

¹ In previous publications from the Imperial Cancer Research Fund and from this laboratory, the inoculation dose, when the needle method is used, has been estimated as 0.01 or 0.02 gram; but such grafts have recently been found, as a matter of fact, to weigh about 0.002 and 0.003 gram respectively.

sections), attention being focused on the stroma reaction, the relative number of lymphocytes, the degree of fibrosis, the vascularity of the surrounding tissues, and the condition of the graft. The results of this examination are arranged and tabulated below (table 1).

Mouse carcinoma 5 was inoculated into 25 rats, and the grafts were removed on the twelfth and thirteenth days after inoculation. Upon removal, 68 per cent of the grafts of this tumor were degenerated. In the degenerated grafts, lymphocytic infiltration and fibrosis existed in about equal degree, though one or the other might predominate in the individual graft. In the grafts in which the tumor was growing, or in which the cells persisted in good histological condition (38 per cent), lymphocytes were scarce and fibrosis was almost absent. These results are capable of two interpretations; (a) either fibrosis and lymphocytic infiltration were preliminary to the death of the graft, or (b) fibrosis and lymphocytic infiltration was a replacement process, occurring after the death of the graft.

Mouse carcinoma 58 was inoculated into 14 rats, and the grafts were removed on the twelfth day after introduction. This tumor in foreign hosts calls forth a tissue reaction of very low grade, characterized by a relative scarcity of lymphocytes, a slight connective tissue response, and a poor blood supply to the surrounding tissues and the graft. While the histologically apparent causes leading up to the death of the graft may possibly be found in the presence of lymphocytes and the fibrous changes, slight though these reactions are, the scanty blood supply appears to be the more important factor. The study shows that a slowly growing tumor, when introduced into an alien host, causes a relatively slight response by the tissues of that animal, and that the fate of the graft is not determined simply by the relative number of lymphocytes and the degree of fibrosis.

Mouse sarcoma 180 was inoculated into 16 rats, the grafts being removed on the tenth and twelfth days of growth. Of the two growing tumors in this series, one was surrounded by but few lymphocytes, while the other was surrounded by a

large number of these elements. In several of the rats in which the tumors failed to grow, lymphocytes were few in number about the degenerated graft. In these animals, fibrous changes about the degenerated tumor were slight.

Rat sarcoma 7 was inoculated into 21 mice, the grafts being removed ten, thirteen, and fourteen days after inoculation. This tumor produces in twelve to fourteen days a very mild reaction, characterized by a small number of lymphocytes in the immediate neighborhood of the graft, and an absence, or only a slight grade, of fibrosis. The tissue about the graft was very vascular and generally edematous. About the blood-vessels in this tissue, at a distance from the graft, the lymphocytes were more abundant. It seems scarcely permissible to attribute the death of the graft to the action of the lymphocytes, unless it be conceived that the lymphocytes exert their lethal action at a distance. In one animal, in which the graft was much degenerated, showing but few surviving sarcoma cells, no lymphocytes could be detected in the tissues or in the immediate vicinity of the tumor. In the majority of the other animals, the extent of degeneration of the tumor cells can not be explained by the number of lymphoid cells, as they were few, even when degeneration was marked. Neither can death of the graft be referred to the activity of the fibrous tissue, for in cases where degeneration of the graft was extensive or even complete, fibrosis was absent or only beginning. A more plausible explanation of the death of the graft, based solely upon the microscopical picture and excluding cytolysis for the reasons given in another paragraph, is found in the disturbance of nutrition due to the edema of the tissues, and consequent interference with the blood supply.

In a further series of inoculations, the following results were obtained. Rat tumor 7, which was inoculated into 24 mice, produced at the end of fourteen days 5 growing tumors, the largest measuring 3 by 8 mm., and 9 degenerated tumors with surviving tumor cells. Mouse tumor 180 was inoculated into 109 young, and very young rats, and gave rise on the fourteenth day after inoculation to 9 tumors, the largest being 7 by 11 mm.

The same strain of tumor was inoculated into 200 older rats, of which 7 had tumors on the thirteenth day, the largest measuring 6 by 10 mm.; on the twenty-first day this tumor measured 5 by 7 mm. There was no evidence that young rats, two to six weeks old, offer a better soil for heteroplastic tumors than do animals three to five months old. The grafts did not grow so well in sick as in healthy animals.

The following conclusions may be drawn from this study. While the causes leading to death of heteroplastic grafts may, perhaps, be attributed to the combined action of lymphocytes and connective tissue, one or the other exerting the predominating action, they are not always determined by the number of lymphocytes or the degree of fibrosis. With certain tumors (sarcoma), death of the graft is not due to fibrosis and cannot be attributed to the lymphocytes, unless it be assumed that these cells act through a distance by the production of some toxic agent (cytolysin or cytotoxin) inimical to the tumor cells. Tumors introduced into animals of foreign species elicit at first a reaction of very much the same character as that produced in homologous animals. Ehrlich has shown that certain tumors survive for only about a week in the tissues of an animal of alien species and until recently this fact was held to be applicable to heterologous grafts in general. W. E. Bullock (4), however, has demonstrated that they can be kept alive in foreign tissues for nearly three weeks, and that if removed and transplanted into other animals of the same foreign species before regression sets in, can be propagated for several generations. Bullock's observation confirms a previous observation of Lewin (5), who was able to grow a rat sarcoma in a mouse for three weeks. Tumor strains show wide differences in the character of their growth in foreign hosts (see table 1), and Bullock's successful results may be explained by the choice of tumor used for the inoculation.

In another connection (6), we have demonstrated that removal of the spleen has no effect upon the fate of a homologous graft. Since the publication of that article, Murphy and Morton (2) have asserted that heterologous tumor grafts grow better

TABLE 1

TUMOR	STROMA REACTION	LYMPHOCYTES	FIBROSIS	VASCULARITY	CONDITION OF GRAFT
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>		<i>per cent</i>
Mouse carcinoma 5.....	Positive 32	Few 20	Absent 8	Moderate	Growing 16
	Negative 68	Moderate 48	Slight 12		Persistent 16
		Abundant 32	Moderate 56		Degenerated 68
Mouse carcinoma 58.....	Positive 7	Few 21	Slight 86	Poor	Growing 7
	Negative 79	Moderate 79	Moderate 14		Degenerated 93
	Negative? 14				
Mouse sarcoma 180.....	Positive 12	Few 19	Slight 25	Moderate	Growing 12
	Positive? 6	Moderate 6	Moderate 25		Degenerated 88
	Negative? 82	Abundant 75	Extensive 50		
Rat sarcoma 7.....	Positive 28	Very few or none 62	Absent 67	Marked	Growing 28
	Positive? 43	Few 24	Slight 33		Persistent 43
	Negative? 29	Moderate 14			Degenerated 29

In classifying the stroma reaction as "positive" or "negative," it may be maintained that the time interval after inoculation is (10-14 days) in some animals long enough to permit a stroma reaction occurring and subsiding before examination. The truth of this possibility cannot be denied.

and survive for a longer period of time in animals which have been exposed to x -rays, attributing this to damaged lymphoid tissues. With the idea of impairing the lymphocytic processes of the body, the spleens were removed from 132 animals either before or after inoculation with alien cancer. One hundred and fifty-six normal animals served as controls. The tumors used were mouse carcinomata 5 and 58, mouse sarcoma 180, and rat sarcoma 7. From twelve to fourteen days after inoculation, the tumor grafts were removed and studied microscopically.

TABLE 2

TUMOR	NUMBER OF ANIMALS	TIME OF SPLEEN REMOVAL	GRAFT EXCISED IN DAYS	NUMBER OF TAKES	NUMBER SHOWING SURVIVING TUMOR CELLS	MAXIMUM SIZE OF TUMOR
5	7	6 days post inoculation	12	0	1	0.5 by 1 mm.
5	30	Not removed	14	0	1	
58	22	5 and 6 days post inoculation	12 and 13	0	2	
58	19	Not removed	12	1	0	
7	47	1 day before inoculation	12 and 13	0	10	
7	47	Not removed	12 and 13	0	6	10 by 12 mm. on 12th day
180	56	1 day before inoculation	10, 12 and 13	5	3	
180	60	Not removed	10, 12 and 13	2	0	
						3 by 4 mm. on 12th day

ally. As shown in table 2, the spleen-free group gave a total of five growing tumors, and 16 growths more or less degenerated, but containing living tumor cells. In the control group, there were three growing nodules and seven degenerated ones with a few living cells. The growths averaged larger in the spleen-free than in the control animals, and the largest tumor (measuring 10 by 12 mm. on the twelfth day) was found in a spleen-free animal. Since these results are within the bounds of individual variations, it may be concluded that removal of the spleen has no appreciable influence upon the receptivity of an

animal for heteroplastic grafts, and that the growth of these grafts is not favored thereby.

Russell, Da Fano, and Bashford and Russell have shown that one inoculation of a heterologous tumor induces an immunity in the host toward a subsequent graft of the same strain of heterologous tumor. Bashford and Russell have specifically stated that this immunity is one directed against the foreign tumor merely as a foreign tissue and not in any sense as a neoplasm. We repeated these experiments, using splenectomized as well as normal animals, and various types of heterologous tumor for the second inoculation.

Twenty-one spleen-free and thirty-two control rats which had been inoculated with mouse tumor 180 (a sarcoma), and in which the tumors had disappeared, were reinoculated with mouse carcinoma 5. The results were negative.

Twenty-seven spleen-free and 34 control mice which bore no tumors after inoculation with rat sarcoma 7, were reinoculated twenty-one days later with the Flexner-Jobling carcinoma. No growing tumors were observed after eleven days, but a few of the grafts contained persisting cancer cells. Ninety-six rats inoculated with mouse sarcoma 180, 6 of which had tumors measuring 4 by 4 mm. or over, were reinoculated with the Ehrlich mouse sarcoma twenty-three days subsequent to the first inoculation. One had a growing tumor 6 by 9 mm. twelve days after inoculation and 4 by 6 mm. a week later. This tumor was found in an animal which had produced a growing tumor on the first inoculation with tumor 180. One hundred and eighty normal rats unsuccessfully inoculated with mouse sarcoma 180 were reinoculated with mouse sarcoma 37. Twenty-five bore tumors (see Chart 1) which in twelve days reached their maximum size and gradually receded thereafter; the largest tumor attained the size of 11 by 16 mm. The longest period of time in which cancer cells persisted in the tissues of an alien species was fifty-four days. This occurred after the inoculation of an emulsion of rat carcinoma 9 into a mouse. Figure 1 illustrates the persisting carcinoma cells; figures 2 and 3 show for comparison a larger field of tumor 9

taken from a stock series, and a section of mouse breast. It will be noted that the tumor cells are in better condition than the stroma, the latter showing marked hyaline degeneration.

Bashford and Russell, as a result of their investigations, consider immunity to heterologous tumors as a manifestation of immunity to a foreign tissue and not to tumor, and according to their view point, we should expect immunity to a second

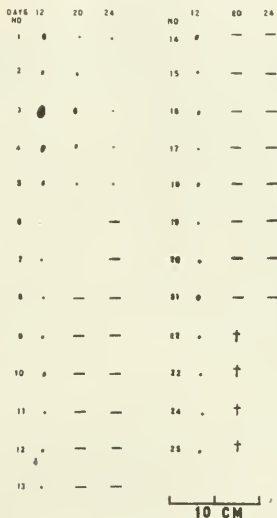


CHART 1

inoculation of heterologous tumor irrespective of the type of tumor used. The results of a second inoculation of heteroplastic tumor do, in fact, agree with the results recorded by the investigators quoted when sarcoma inoculation is followed by carcinoma inoculation, but they do not appear to harmonize when sarcoma inoculation is followed by sarcoma inoculation. Several explanations may be given for the diver-

gent results; they may be due to a difference in the growth energy of the two tumors at the time that they were used; or to a difficulty in producing an immunity against these sarcomata (7); or finally, it is possible that the dosage of the initial inoculation (0.003 gram) was insufficient to cause immunity against the second inoculation.

SUMMARY

While the death of some heteroplastic tumor grafts may, perhaps, be attributed to the action of lymphocytes and connective tissue, there is no histological proof that it is always determined by these factors.

Tumors elicit in foreign species a reaction of much the same character as that produced in homologous animals.

Removal of the spleen has no influence upon the receptivity of an animal toward heteroplastic tumor grafts.

Splenectomy does not favor the growth of heteroplastic tumor grafts.

One inoculation of a heterologous tumor does not always render an animal immune to the temporary growth of a subsequent heterologous graft.

REFERENCES

- (1) DAFANO, C.: *Ztschr. f. Immunitätsforsch. u. exper. Therap.*, 1910, v, 1; Fifth Sci. Rep., Imperial Cancer Research Fund, London, 1912, 57.
- (2) MURPHY, J. B., AND MORTON, J. J.: *Jour. Exper. Med.*, 1915, xxii, 204; *Proc. Nat. Acad. Sc. Bull.*, 1915, i, 435; *Jour. Exper. Med.*, 1915, xxii, 800.
- (3) LOEB, LEO.: *Jour. Am. Med. Assn.*, 1915, lxiv, 726.
- (4) BULLOCK, W. E.: *Lancet*, London, 1915, i, 701.
- (5) LEWIN, C.: *Ztschr. f. Krebsforsch.*, 1907-08, v, 16; *ibid.*, 1908, vi, 306.
- (6) ROHDENBURG, G. L., BULLOCK, F. D., AND JOHNSON, P. J.: *Studies in Cancer and Allied Subjects*, George Crocker Special Research Fund, N. Y., 1913, iii, 87.
- (7) TSURUMI, M.: *Jour. Path. and Bacteriol.*, 1915-16, xx, 76.

PLATE 1

FIG. 1. GRAFT OF RAT TUMOR 9, PERSISTING IN MOUSE TISSUE FOR 54 DAYS.
× 250



PLATE 2

FIG. 2. NORMAL GROWTH OF RAT TUMOR 9, FOR COMPARISON WITH FIGURE 1.
× 250

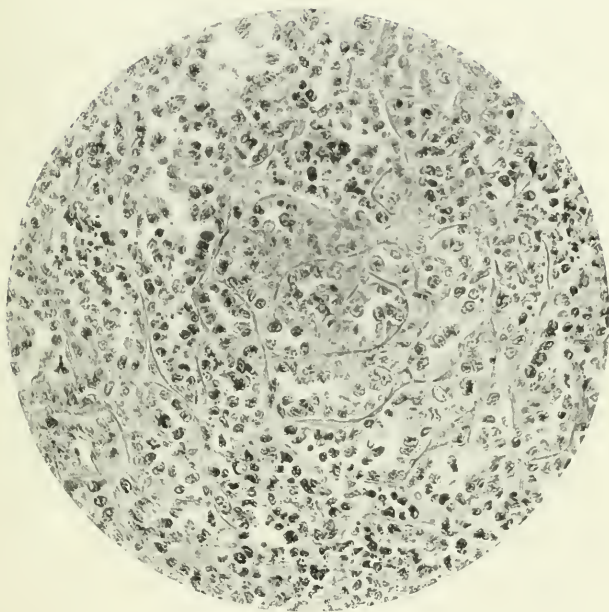


PLATE 3

FIG. 3. NORMAL MOUSE BREAST. $\times 250$



SIGNIFICANCE OF THE CARTILAGE IN A CARCINO- CHONDROSARCOMA OF THE MOUSE

WILLIAM H. WOGLOM

*From Columbia University, George Crocker Special Research Fund, F. C. Wood,
Director*

Received for publication, July 9, 1917

A sarcomatous transformation of the stroma (1) in spontaneous and transplantable carcinomata of the mouse is not uncommon. In the rat, the cases are not so numerous, but this may be due only to the fact that comparatively few transplantable carcinomata have been found in this species; certainly its tissues are far from unlikely to give rise to sarcoma under the ordinary conditions of life to which these rodents are subjected, as is shown by the work of Bullock and Rohdenburg (2), and of other less recent authors. Whatever be the reason, the number of carcinosarcomata reported in the rat amounts to but three (3, 4, 5).

One such tumor has been discovered in the dog by Wells (6), and in man they are so extremely rare that up to 1908 Herxheimer (7) had been able to find only some twenty authentic cases in the entire literature, while six years later Saltykow (8) mentioned "about twenty-five," to which he added three more.

Although the transformation is regarded by most authorities as a sarcomatous change in the connective tissue framework of the tumor, this opinion is not yet universal, at least so far as neoplasms of the mouse are concerned. What these spindle cells actually are, and why they appear as they do in a few neoplasms, is an unsolved riddle, and one of the most important in pathology; for if they be sarcoma, then the unique opportunity has been given of observing the neoplastic process from its very inception.

Most of the authorities who regard the alteration in question as a true sarcomatous change, suggest that it arises in obedience

to some special sort of stimulation exerted upon the stroma by the epithelial portion of the tumor, though what the nature of the exciting agent may be is not even suspected; some refer to it vaguely as chemical, and there are not wanting those who would attribute the alteration to a parasite living in symbiosis with the carcinoma cell. The hypothesis that the stroma becomes sarcomatous in consequence of repeated transplantation was seen to be untenable as soon as the transformation was discovered in a spontaneous tumor (9), while the suggestion that the cellular connective tissue between the alveoli is merely granulation tissue fell to the ground with the demonstration that it can be transplanted (10).

Still other explanations have been advanced in elucidation of the sarcomatous change. Thus Ribbert (11) asserts that no irritation by the parenchyma would elicit sarcomatous transformation unless the stroma were especially susceptible to it; now since the transplantable tumors of the mouse, he believes, are not true carcinomata at all, but rather a species of neoplasm allied to the cylindromata, they must have a stroma which properly belongs to them, or in other words, one springing from a connective tissue embryonal remnant displaced coincidentally with that other one which gives rise to the epithelial portion of the growth; this stroma, transplanted with the parenchyma at each inoculation, becomes continually more independent until finally it develops into sarcoma. His hypothesis of a mixed epithelial and connective tissue embryonal remnant, he thinks, will probably best explain carcinoma sarcomatodes in the human subject also, some unknown influence taking the place of transplantation in inciting to malignant proliferation a stroma already so predisposed.

All the hypotheses so far discussed have this much, at least, in common, that all concede the spindle-shaped cells between the alveoli to be derivatives of the connective tissue. A few observers, however, and notably Krompecher (12), deny any such origin, and insist that these are epithelial elements which have assumed a spindle shape; other writers, it is true, have suggested such a change in morphology as an alternative hypo-

thesis, but none has espoused it so warmly as Krompecher. His view, which he bases upon the zoölogical investigations of others as well as upon his own oncological research, may be summarized as follows: A direct transformation of epithelium into connective tissue takes place normally in the embryo and among the lower vertebrates. The conditions necessary for this change are a heightened proliferative activity in the epithelium and an appropriate plasticity in the stroma, both of which exist not only in the embryo and the lower vertebrates, but in carcinomata with "hyaloid" or myxomatous stroma. Under such circumstances, the epithelium becomes frayed out until its cells resemble exactly those of the connective tissue, as to both nucleus and protoplasm. Furthermore, collagen fibrils have been demonstrated in epithelial cells that have assumed the spindle shape, and even the production of cartilage by ectoderm has been described by several writers.

If, continues Krompecher, epithelium can be transformed into connective tissue, as all these observations appear to imply, it is difficult to refute the hypothesis that in carcinosarcomata the sarcomatous portion develops from the carcinomatous; and he terminates his article with the conclusion that while epithelium is ordinarily evolved from epithelium, and connective tissue from connective tissue, the direct metaplasia of epithelium into connective tissue (or of carcinoma into sarcoma) cannot be gainsaid, and, indeed, under suitable conditions, is not rare. Few pathologists are willing; however, to transfer to adult human cells the findings of the embryologist and the zoölogist, at least until further experience has demonstrated that a parallel may safely be drawn.

That there does occur in the mouse a type of new growth in which the epithelium assumes a spindle shape, can scarcely be denied. Apolant (13) has described a transplantable tumor of this sort, and three similar spontaneous neoplasms have come under the writer's observation at the Crocker Fund, two of which are here reproduced. Still, this may not be the whole story. It will be observed from figures 1, 2, and 3 that the transition to the spindle shape is gradual, and from figure 4

(it is not so in all such tumors) that the change has occurred toward the center of the alveoli; quite the opposite appearance characterizes the condition ordinarily believed to represent a sarcomatous transformation of the stroma, the line of demarcation being there abrupt and the spindle cells lying outside the alveoli, from which they are often separated, indeed, by characteristic halos of clear cells (14). All this suggests that there are two distinct varieties of tumor containing spindle cells, one (pure carcinoma) in which these elements are of epithelial, and another (carcinosarcoma) in which they are of connective tissue origin. Yet morphological evidence is, after all, apt to be misleading, and it is fortunate that other proofs in favor of this view are not lacking.

It is the province of this communication to offer certain new observations in favor of the hypothesis that the spindle-shaped cells in the type of growth ordinarily called carcinosarcoma are actually of connective tissue origin; it must nevertheless be admitted at once that the evidence is not actually conclusive, though it may be regarded as highly suggestive unless one wishes to assume, with Krompecher, that epithelium may be able to produce connective tissue elements.

In a transplantable mouse carcinoma (no. 48), which possessed temporarily the power to produce the sarcomatous transformation in its stroma, cartilage¹ (fig. 5) was discovered in at least three of the four mixed growths in the second generation (2A); as only a very small piece of the fourth tumor was available for examination, it cannot be said definitely that this growth contained no cartilage. The three tumors were 75, 177, and 205 days old respectively, while that one in which it was not found was 76 days of age. Cartilage appeared once more, in a 53-day old growth of the third generation.

The readiest explanation of such a finding is, of course, that a portion of costal cartilage had become involved by the tumor during its growth, and had been removed with it. Serial sections, however, proved conclusively that this was not the case; many independent islands of cartilage, some composed of only

¹ Kaufmann (Lehrbuch Path. Anat., Berlin, 1911, ii, 1089) has reported, as a unique finding, a chondrosarcomacarcinoma of the mamma in the human subject.

a few cells (fig. 6), lay scattered about through the stroma, and, furthermore, there was a very gradual transition between these cartilaginous areas and the surrounding sarcoma (fig. 7). No course remains open, therefore, but to regard this cartilage as a product of the spindle-shaped cells surrounding it.

The question immediately arises, then: What sort of cells were these? Krompecher (15) quotes observations by a number of investigators which seem to suggest that products of the various germinal layers cannot be sharply distinguished one from the other, and especially germane to the present article is the production of cartilage by the ectoderm, a process which has been observed by Platt and others. But, as Krompecher himself points out, the transformation of one type of tissue into another, and particularly of epithelium into connective tissue, cannot be directly proved in postembryonal life; here the problem can be solved only indirectly by the study of transitional stages and by the help of analogy.

Since it is not definitely known whether or not epithelium which has once matured can produce cartilage, and since, on the contrary, it is known that connective tissue tumors are able to form cartilage, it seems safer to assume, at least for the present, that any neoplasm which has produced cartilage must have contained connective tissue elements. If this hypothesis be sound, then the spindle cells in tumor 48, and probably those also in other neoplasms of its class, were of connective tissue derivation, and the name carcinosarcoma is justified.

Evidence somewhat resembling that offered in the present paper has been adduced by Haaland (16), who discovered intra- and intercellular fibrils in the sarcomatous parts of mixed neoplasms, and, more recently, also, by Slye, Holmes, and Wells (17), who regard the presence of myxomatous degeneration in one of their tumors which resembled the mixed growths, as suggestive of sarcoma.

As cartilage was never found in the stroma of pure carcino-mata in tumor 48, it is obvious that interpolation of the sarcomatous transformation was one of the conditions necessary for the emergence of this metaplasia. What the others were, however, it is impossible to say. The fact that the connective tis-

sues of three mice, all of one breed, showed cartilaginous differentiation, makes it impossible to dismiss, as a second factor, some such predisposition to cartilaginous metaplasia in this strain as characterizes, for example, the dog's mamma, though the explanation may be regarded as improbable. The reappearance of cartilage in the following (third) generation, in a mouse of different breed, is deprived of any significance by the fact that this generation (3A) was transplanted from that (2A) in which the cartilage arose; the existence of cartilage in it, therefore, may have been due merely to the transplantation from the preceding generation of sarcoma cells able to differentiate in this direction. A third factor, and the only remaining common one, was the presence of carcinoma 48, the parenchyma of which seems to have had the power not only to bring about the sarcomatous change, but to determine differentiation into cartilage in the sarcoma so educed. If this neoplasm ever regains the power to induce sarcoma, it will appear, without much doubt, that the emergence of cartilage is associated with the parenchyma rather than with the stroma, since it is already known that the carcinomata exert other influences, quite as remarkable, upon their stromas.

It seems more probable that the cartilage emerged by differentiation of the connective tissues, than that embryonal remnants of cartilage were present; for while the connective tissues of the mouse may, perhaps, contain embryonal rests, misplaced when the various cartilages were laid down and potentially able to form cartilage under pressure of a suitable agent such as the parenchyma of carcinoma 48 appears to have been, their existence has not been proved. In favor of the differentiation hypothesis, on the other hand, stands the fact, already known, that certain carcinomata in man cause the production of bone in their stroma, while certain other ones in the mouse compel the elaboration of a highly vascular connective tissue scaffolding. If bone or blood-vessels, it may well be asked, why not cartilage?

Hence the preferable explanation of the occurrence of cartilage in tumor 48 is to ascribe to the parenchyma of this neo-

plasm the power to determine cartilaginous differentiation in a sarcomatous stroma. A similar hypothesis has been advanced in the description of a spontaneous chondroosteosarcoma of the mouse mamma by Murray (18), who, like the present writer, regards as unnecessary the assumption of a congenital foundation for the presence of cartilage in spontaneous mammary tumors, preferring to ascribe it to postnatal differentiation.

SUMMARY

The presence of cartilage in the sarcomatous portion of a carcinosarcoma in the mouse strongly suggests that the sarcomatous part was of connective tissue, rather than of epithelial origin.

REFERENCES

- (1) Literature reviewed by Woglom in "The Study of Experimental Cancer," Columbia University Press, New York, 1913.
- (2) BULLOCK, F. D., AND ROHDENBURG, G. L.: Jour. Cancer Research, 1917, ii, 39.
- (3) FLEXNER, S., AND JOBLING, J. W.: Proc. Soc. Exper. Biol. and Med., 1906-1907, iv, 12; 1908, v, 52, 91; Monographs on Medical and Allied Subjects, Rockefeller Institute, N. Y., 1910, No. I, p. 1.
- (4) LEWIN, C.: Ztschr. f. Krebsforsch., 1907-1908, vi, 273.
- (5) LOEB, LEO: Amer. Jour. Med. Sc., 1903, cxxv, 243.
- (6) WELLS, H. G.: Jour. Path. and Bacteriol., 1901, vii, 357.
- (7) HERXHEIMER, G.: Ziegler's Beitr., 1908, xlv, 150.
- (8) SALTIKOW, S.: Centralbl. f. allg. Path., 1914, xxv, 419.
- (9) EHRLICH, P., AND APOLANT, H.: Berl. klin. Wehnschr., 1907, xlv, 1399.
- (10) BASHFORD, E. F., MURRAY, J. A., AND HAALAND, M.: Berl. klin. Wehnschr., 1907, xlv, 1238.
- (11) RIBBERT, H.: Das Karzinom des Menschen, Bonn, 1911, p. 376.
- (12) KROMPECHER, E.: Beitr. z. path. Anat. u. z. allg. Path., 1908, xlv, 88.
- (13) APOLANT, H.: Arch. f. mikroskop. Anat.: 1911, lxxviii, 144.
- (14) HAALAND, M.: Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 196.
- (15) Krompecher, E.: l. c.
- (16) HAALAND, M.: Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 250.
- (17) SLYE, M., HOLMES, H. F., AND WELLS, H. G.: Jour. Cancer Research, 1917, ii, 20.
- (18) MURRAY, J. A.: Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 78.

PLATE 1

FIG. 1. SPONTANEOUS MOUSE CARCINOMA $\left(\frac{646}{0}\right)$ WITH SPINDLE EPITHELIUM.
× 250

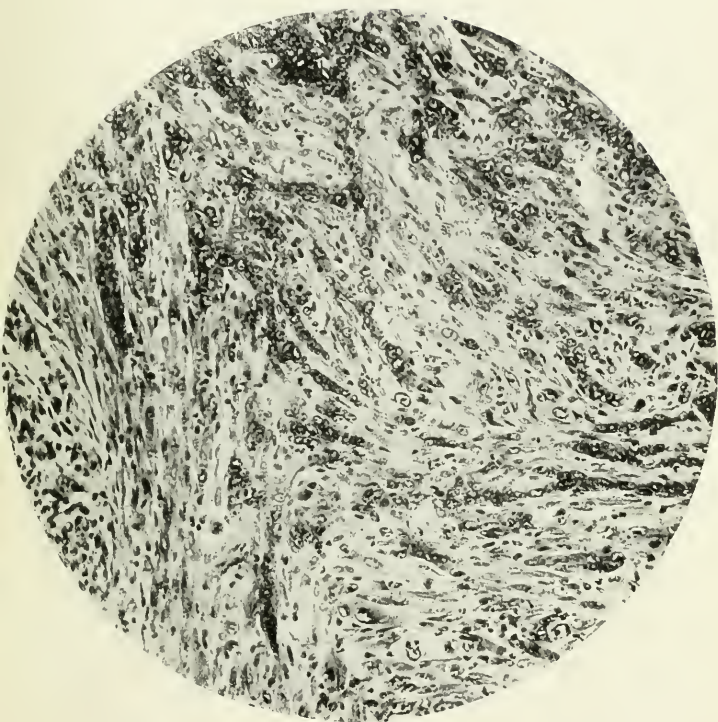


PLATE 2

FIG. 2. SPONTANEOUS MOUSE CARCINOMA $\left(\frac{936}{0}\right)$ WITH SPINDLE EPITHELIUM.
× 250

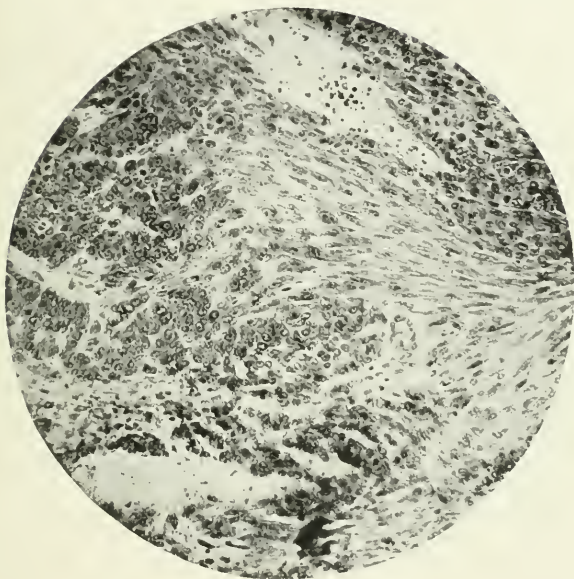


PLATE 3

FIG. 3. HIGH POWER VIEW OF CENTRAL PORTION OF FIGURE 2. $\times 480$

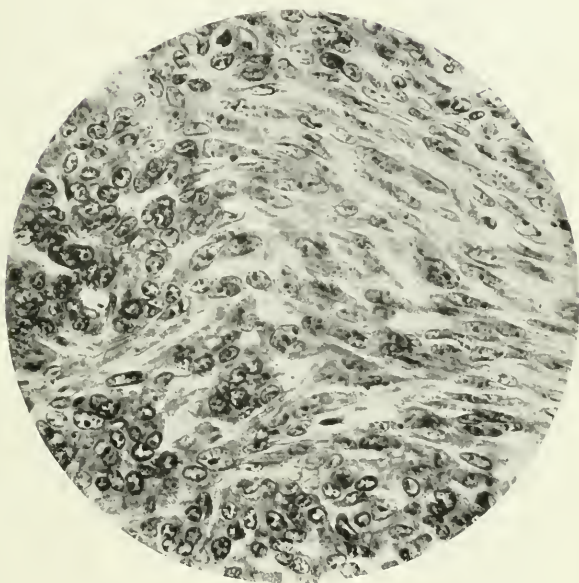


PLATE 4

FIG. 4. SPONTANEOUS MOUSE CARCINOMA $\left(\frac{636}{0}\right)$ WITH SPINDLE EPITHELIUM
 $\times 80$

FIG. 5. TUMOR 48. CARTILAGE IN A MIXED 205-DAY OLD GROWTH OF THE SECOND
(2A) GENERATION. $\times 75$

FIG. 6. SMALL ISLAND OF YOUNG CARTILAGE IN SECOND GENERATION (2A)
TUMOR 48. $\times 400$

FIG. 7. GRADUAL TRANSITION BETWEEN SARCOMA AND CARTILAGE IN SECOND
(2A) GENERATION OF TUMOR 48. $\times 150$



FIG. 4

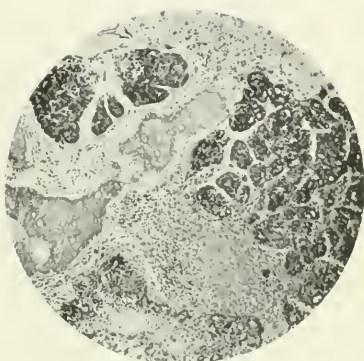


FIG. 5

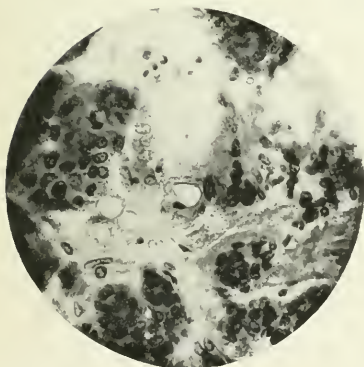


FIG. 6

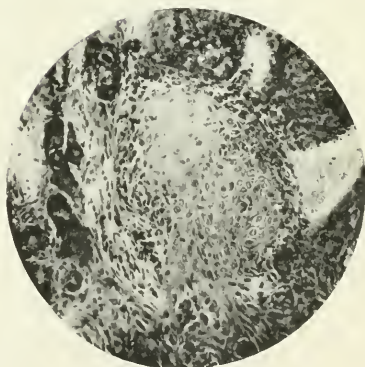


FIG. 7

GROWTH OF TUMORS IN THE CHICK EMBRYO

HOLLAND N. STEVENSON

*From Columbia University, George Crockcr Special Research Fund, F. C. Wood,
Director*

Received for publication, July 30, 1917

When Murphy (1) introduced the chick embryo as a soil for the growth of foreign tissues, it appeared that it might prove to be a medium for the growth of human neoplasms as well as for the transplantable tumors of animals, offering thus a wide field for investigation. This report presents the results of experiments that were undertaken to determine any differences that may occur between tumor growth in the original species and in the chick embryo.

The problem was divided into three parts. The first dealt with the results obtained with tumors of different growth rates, though it included, also, a study of the general characteristics of tumors growing in the chick embryo and the results obtained with human tumors inoculated into this medium. The second problem was a comparison between early stages of tumor grafts in the chick and in the original species. The third dealt with the effect of prolonged residence in the chick upon the growth of a mouse tumor therein, and its behavior when returned to the animal.

In the experiments undertaken the eggs employed were obtained from one dealer, but were not all from the same breed of fowl. It does not seem likely, however, that a difference of breed would have an influence on the growth of tumors from a different class of animal. The eggs were incubated at 39.4°C. and inoculations were made on the fifth to the eighth day of incubation, the eggs having been previously candled and the location of the embryo marked. The technique of the inoculation, similar to that described by Peebles (2) and later developed

by Murphy (1), was as follows: With a small knife a square of about 7 mm. was cut in the shell and carefully removed. The vitelline membrane was then torn with sterile forceps and the tumor graft inoculated with a pair of curved forceps through this opening upon the underlying allantois. The fragment of shell was then replaced and sealed with paraffin. After inoculation, the eggs were returned to the incubator and kept there until the seventeenth or eighteenth day of incubation, when they were removed and examined for the graft.

It was found that about 35 per cent of the embryos survived the operation. The number bearing tumors varied with the growth used and also with the series. For example, with the Ehrlich sarcoma the number of takes was usually between 70 and 80 per cent, while with the Crocker mouse carcinoma 11 only about 50 per cent of takes were found. From this it is seen that tumors grow in the membranes of about 30 per cent of the total number of eggs used.

GROWTH RATE

The time factor is of the greatest importance for tumors growing in the egg. In animals many transplantable tumors do not appear until two or three weeks after inoculation, and occasionally even longer; now when one considers that a tumor has at most thirteen days, and usually less (Murphy (3)), to grow in the chick embryo, it is obvious that unless slowly growing tumors proliferate much more rapidly here than in the animal, only a very small nodule, or none at all, will be found at the conclusion of this period. The time factor is especially concerned where the attempt is made to grow human tumors in the egg, for these increase in size even more slowly than transplantable animal tumors.

In the experiments undertaken to discover what bearing growth rate in the animal has upon tumor growth in the chick embryo, six transplantable tumors were used. These included three mouse tumors, the Ehrlich sarcoma, the Crocker carcinoma 11, and the Twort carcinoma; two rat tumors, the Im-

perial Cancer Research Fund carcinoma 9 and sarcoma 16; and one guinea-pig sarcoma, Crocker 1.

In the animal, the Ehrlich sarcoma and carcinoma 11 are tumors of rapid growth, usually giving rise to a nodule the size of a pea within a week; the latter, however, grows somewhat more slowly than the former. The Twort carcinoma grows more slowly than no. 11, and does not generally appear until the third week. The two rat tumors are both slow-growing, but of slightly different characteristics; sarcoma 16 appears usually by the third week and enlarges fairly rapidly, while rat carcinoma 9 often appears by the second week, but enlarges more slowly. The Crocker guinea-pig tumor 1 is a fibrosarcoma that appeared in the first generation only after six to eight months, and in later series after three months or more. These characteristics are, of course, general and subject to variation, but they give a fair idea of the growth rate of these tumors in the animal body.

The tumors were inoculated into series of eggs in uniform dosage—approximately 0.003 gram in each instance. It was impossible to inoculate all the tumors at once, but the factors of soil, temperature, and technique were not varied throughout the experiment.

The following series of experiments has been chosen at random from among many others, all of which gave the same result.

Of 36 eggs inoculated with the Ehrlich sarcoma, 8 were alive when opened ten days later, and of these 4 bore tumors. The grafts had grown well and averaged 5 mm. in diameter, being almost spherical in shape. Microscopically, they exhibited the characteristics of the Ehrlich sarcoma in a healthy growing condition.

Carcinoma 11 was inoculated into 32 eggs in a similar way. Nine days later, 7 of the eggs were found with living embryos, of which 5 bore tumors. These growths averaged between 3 and 4 mm. in diameter, were generally spherical in shape, and showed microscopically the characteristics of this tumor when in a healthy growing condition.

The Twort mouse carcinoma was inoculated into 63 eggs.

Ten days later 6 eggs were found to contain living embryos, and of these 3 had small areas on the membranes, not more than 2 mm. in diameter. These areas were not spherical but flat, and it was difficult to tell by the naked eye whether or not they were composed of growing tissue. Microscopic examination proved, however, that all three areas contained healthy growing tumor.

Rat carcinoma 9 was inoculated into 53 eggs in the usual way. Ten days later the eggs were opened, and 15 living embryos were found, of which 6 bore what were evidently grafts. The largest of these was 6 mm. in diameter and 2 mm. in thickness; the others were about 3 mm. in diameter, not spherical but flat, and less than 2 mm. thick. Sections showed all the areas to contain healthy growing tumor.

Rat sarcoma 16 was inoculated into 23 eggs. Ten days later the eggs were opened and 5 were found to contain living embryos. Of these 2 showed small translucent areas on the membranes, one 1.5 mm. in diameter and the other 1 mm. in diameter. Both were only slightly elevated. On section they were proved to contain growing tumor tissue.

With the Crocker guinea-pig sarcoma 1, 57 eggs were inoculated in the usual way. Ten days later there were discovered 7 areas which appeared to be thickenings of the membrane. One of these on section showed the presence of a few tumor cells which, although evidently living, showed no signs of growth.

These experiments clearly demonstrate that the growth rate of a tumor in animals is an important factor in determining the size which the growth will attain in the chick embryo. With the two tumors which grow rapidly, the Ehrlich sarcoma and carcinoma 11, good sized tumors resulted in the chick. With the three tumors that grow more slowly in the animal, Twort, rat 9, and rat 16, much smaller growths resulted in the chick with the same dosage, in the same time and under similar conditions. The outcome with the guinea-pig sarcoma, the most slowly growing of all, in the animal, is even more distinct in this respect than in the case of any of the other tumors. This tumor gave no growth at all in the chick, although several series of inoculations were made.

In the discussion of these results some points of interest may be brought up. The first is, that tumors growing in the chick embryo preserve approximately the same growth rate that they exhibit in the original animal. This has an important bearing upon the use of the chick as a soil, because, for experiments that must continue for more than one generation, this medium becomes limited to the more rapidly growing tumors. Its limitations as a culture medium become even more obvious when one considers that of the fertile eggs inoculated, less than 30 per cent bear tumors, and that the resulting grafts are so small in the case of slowly growing tumors, that by the second generation a sufficiently large number of eggs could not be inoculated. The result would be that after a few generations the experiment would be automatically terminated by lack of tumor.

Another interesting finding is that the chick embryo is not a more favorable growth medium for the animal tumors than the healthy animal in which it is native. If it were, one might expect to see the slowly growing tumors much larger than they are in the animal within the given time. This is not the case, however, for they appear to attain in the chick about the same size that they do in the animal after proliferating for an equal period. This apparently eliminates any possibility of stimulation or of retardation of the growth in the chick, so far as the methods employed can determine.

During the foregoing experiments, many microscopical features were noted, which may be of interest here. In general one may say that tumors growing in the chick embryo retain the structure which characterizes them in the animal, except that their stroma is now derived from the chick.

Sarcoma cells are seen growing out singly from the graft, and mesenchyme cells and blood-vessels from the chick penetrate it. The sarcoma cells appear to be larger, but this is probably due to the lower tissue pressure in the chick; that is, the tissue is looser and the cell has more space in which to expand. Many mitotic figures in all stages of division are present throughout the tumor.

The carcinomata preserve the alveolar or glandular arrange-

ment and their cellular elements have the same appearance as in the animal. Between the groups of tumor cells extend the connective tissue and blood-vessels of the chick. The carcinoma cells do not appear so definitely enlarged as those of the sarcomata, mainly because they retain their group formation and are consequently crowded by one another. Near the edge, where at times they may be seen extending outward in groups of two or three, they are apt to appear larger than towards the center of the tumor graft. Mitoses are not quite so frequent as they are in the sarcomata, but are nevertheless numerous.

A biological characteristic of considerable importance is the preservation in the chick of the angioblastic tendencies of a tumor, one instance of which seems especially worth recording. Carcinoma 11 was originally a hemorrhagic growth, and this characteristic reappears occasionally in its routine passage through mice. Such a neoplasm contains large areas of hemorrhage, as well as hemorrhagic cysts, and these features characterized one of the nodules which developed in the chick after inoculation with tumor 11. The spaces, however, were, of course, filled with chick blood corpuscles, and the walls of the spaces composed of chick connective tissue and, so far as could be determined, lined by chick endothelium.

The reaction on the part of the connective tissue and wandering cells around the tumors growing in the chick embryo varies somewhat with each graft. The connective tissue reaction at times is dense, but usually it is delicate and quite typical of embryonic connective tissue. As for the wandering cells, there may be few or many. In some sections, they gather in groups around the blood-vessels. These cells, practically all of which were of the myeloid or granular leucocyte series, were present around all the tumor grafts, the only variation being found in the number present.

HUMAN TUMORS IN THE CHICK EMBRYO

Although the results following the inoculation of human tumors into the chick embryo were not entirely conclusive, it seems advisable to record them in this connection. The

material was obtained as fresh as possible from a hospital nearby, and the inoculations were made in the customary way. The dose was 0.003 gram. It was found impossible always to have eggs at the same age for each tumor, but they were used on, or near, the seventh day of incubation. Eight human tumors, and a metastatic nodule from one of them were inoculated into 350 fertile eggs. The following types of neoplasm were employed: Scirrhus carcinoma of the breast; medullary carcinoma of the breast (two); metastasis from a medullary carcinoma of the breast; squamous-cell carcinoma of the leg; adenocarcinoma of the breast (two); carcinoma of the testicle; chorioepithelioma of the uterus.

No growth resulted from these inoculations; in a few sections, cells that were evidently those of the human tumor introduced were found, but these were often multinucleated and showed no evidence of mitoses. These cells, therefore, were probably not growing, although the possibility that they might have begun to proliferate at some later time is not to be lost sight of.

The spontaneous tumors of mice resemble human tumors more closely than the transplantable ones and it may be of interest to mention here the results obtained with them. A few spontaneous mouse tumors were inoculated into eggs; some of these grew and some did not. The tumors resulting from those that grew were small and gave little promise of prolonged propagation in the chick embryo.

COMPARISON OF EARLY STAGES

In order to determine what difference might occur between the progressive growth of a tumor in the chick embryo and in its original species, a study was made of growing tumors in their early stages in the chick.

Two growths were used, the Ehrlich mouse sarcoma, and the Crocker mouse carcinoma 11, and the technique of inoculation was similar to that used in the other experiments. Tumor to the amount of 0.003 gram was inoculated into each egg on the seventh day of incubation, and the grafts removed at each period and serial sections cut of each graft.

The Ehrlich sarcoma, after twenty-four hours' residence on the chick membranes, showed signs of beginning growth. At the periphery of the graft, the tumor cells appeared to be extending out to a slight degree into the chick mesenchyme. The cells towards the center of the graft, however, showed signs of necrosis, and the stroma also was becoming necrotic. At the periphery of the graft, the mesenchyme was sending delicate filaments between the tumor cells. At the end of forty-eight hours, the process of organization of the tumor had progressed much farther. The cells had migrated some distance from the graft, and the mesenchyme and capillaries of the chick had extended toward the center of the tumor. The tumor cells at the periphery were sending out long filaments which extended into the chick tissue, and also connected one cell to another by more or less prominent bridges. The impression was given that these elements at the border formed a syncytium, but this was probably not the case, for it was possible occasionally to make out a cell membrane in some of the border processes. At the end of the third day, the organization of the graft was usually complete. The capillaries and new stroma formed by the chick tissue were seen throughout the tumor. The necrotic tissue at the center of the graft had been replaced, and the tumor had distinctly enlarged. The later stages were merely a continuation of the process described above. Mitotic figures were numerous in the tumor cells and a certain number of wandering cells were always present.

A study of the preparations of tumor 11 showed the process to be similar in general to that described for the Ehrlich sarcoma. The central necrosis was present and the growth of the tumor and of the reacting chick tissues occurred at the same time that they did with the sarcoma. Those acini at the periphery which remained healthy were completely supplied with stroma and capillaries by the end of the second day. The later stages showed the organization of the area of central necrosis. It is interesting to note that, as this area of central necrosis became organized, the tumor acini grew back into it, following the new tissue until the tumor was uniform throughout. This complete

organization is usually seen by the end of the fourth day. Here also, wandering cells were numerous and the tumor cells contained many mitotic figures.

No material difference has been noted between the manner in which these tumors established themselves in the chick and in the mouse. The chick tissues seem to supply the framework necessary for growth just as efficiently as the animal does. One difference, however, is to be noted; the process is apparently completed in the chick in much less time.

THE EFFECT OF PROLONGED RESIDENCE IN THE CHICK

In a previous communication (4) it was pointed out that the Ehrlich sarcoma, after growth in the chick embryo, shows evidence, on its return to mice, of impaired growth power. After one generation (ten days) in the chick, the number of takes in mice and the size of the tumors were both less than in the control series. After two generations in the chick, or twenty days, the number of takes was still less and the resulting tumors even smaller. It was also pointed out that these growths regained their normal growth rate and percentage of takes when they were transplanted back into mice. Upon repeating these experiments, identical results were obtained; but it was also observed that this sarcoma, after growing continuously in the chick for two generations, contained areas of necrosis, a rare finding among neoplasms grown in this medium.

These results all indicated a distinctly unfavorable effect upon tumors proliferating in the chick embryo, and it was of interest to discover whether or not other growths were susceptible to this influence; for it is well known that tumors differ among themselves in their reactions to various agencies. A similar experiment was accordingly carried out with the mouse carcinoma 11, a tumor with a growth rate somewhat slower than that of the Ehrlich sarcoma, and the ability to take in 100 per cent of animals.

The details of the experiments may be given briefly as follows: A healthy growing tumor was selected and 0.003 gram inoculated into each of a series of eggs, and also into mice, ac-

according to the usual technique. At the end of ten days, the eggs were opened under aseptic precautions and the tumor grafts removed. The largest of these were inoculated (0.003 gram) into a new series of eggs and also into mice, and the procedure repeated until the termination of the experiment.

The tumors proliferating in the chick maintained their usual growth characteristics until the fifth generation (fifty days). While the tumors of the fourth generation were as large and as numerous as those of the first, those of the fifth generation were so small that only enough material was obtained for inoculation into the sixth generation of eggs. On opening the eggs of this, the last generation, only a few pinhead nodules were found, too small for inoculation. The experiment was terminated, therefore, from lack of material to continue it, after the tumor had resided sixty-one days in the chick.

The results of the inoculations into mice after the tumors had grown in the chick for varying periods of time, were inconclusive, because after the fourth generation there was not enough tumor to make these implantations. The growths in mice derived from the various generations in the chick showed no constant variation from the controls. Those resulting from the first, second, and third generation were fewer in number and smaller than their controls; but those resulting from the fourth generation were almost as numerous, and of a size equal to the controls. It may be supposed, however, that if mice instead of eggs had been inoculated from the fifth generation, a definite difference between these and control series would have been found. A comparison of the results with the Ehrlich sarcoma and those obtained with tumor 11 brings out considerable difference. The former exhibited impaired growth after one generation and striking impairment after two generations in the chick, while the latter showed no apparent modification after four generations in the chick. The explanation of this is not obvious. It may be due to the fact that the Ehrlich sarcoma is of more rapid growth rate than tumor 11, and is therefore more susceptible to the inhibiting influence.

In a previous report (5) it was pointed out that spleen grafts growing on the allantois of the chick have no influence upon the growth of tumor grafts similarly located. From this finding, and from the fact that foreign tumors grow so well in this medium, it may be assumed that the chick embryo has no active resistance against such tumors until about the eighteenth day, and, furthermore, that active resistance to tumors can not be induced in the chick embryo. If this be true, it is probable that the effect upon a tumor grown for a long time in the chick is produced, not by an active resistance, but because the tumor is unable longer to break down the foreign proteids and convert them to its use. The fact that the tumor of more rapid growth shows this retarding influence first is in favor of this view, for a tumor of this character will need more energy in the same time than one of slower growth. If there is a limit to the energy a tumor can derive from the chick as a foreign soil, which seems to be probable, the tumor of more rapid growth will be the first to manifest exhaustion.

SUMMARY

The growth rate characteristic of tumors in their native species is preserved for one generation in the chick embryo.

During this period there is no evidence of either retardation or stimulation of the growth rate of a tumor in this medium.

Tumors tend to preserve not only their morphology, but their power to recover antecedent characteristics, and they mold the chick tissues as readily as they do those of the animal host.

There is little hope of being able to maintain a tumor of slow growth indefinitely in the chick. Consequently the cultivation of human tumors in the chick embryo for more than a short period of time does not seem likely to be realized.

The organization of tumor grafts in the chick takes place in a manner similar to that in the original species, but the procedure is more rapid.

The chick exerts an unfavourable influence upon tumors growing in it. This is probably due to a lack of suitable nutritive

material rather than to an active immunity. A tumor will grow in this medium only for a limited time, the period depending upon its growth characteristics.

REFERENCES

- (1) MURPHY, J. B.: Jour. Am. Med. Assn., 1912, lix, 874.
- (2) PEEBLES, F.: Arch. f. Entwcklingsmeehn. d. Organ., 1898, vii, 405.
- (3) MURPHY, J. B.: Jour. Exper. Med., 1913, xvii, 482.
- (4) STEVENSON, H. N.: Proc. New York Path. Soc., 1916, xvi, 60.
- (5) STEVENSON, H. N.: Am. Jour. Cancer Research, 1917, ii, 345.

THE PART PLAYED BY THE HYDROXY-BENZOL RADICAL IN THE ACCELERATION OF THE GROWTH OF CARCINOMATA BY CHOLESTEROL AND BY TETHELIN

T. BRAILSFORD ROBERTSON AND THEO. C. BURNETT

From the Department of Biochemistry and Pharmacology and the Department of Physiology, Rudolph Spreckels Physiological Laboratory, University of California

Received for publication, July 30, 1917

I. INTRODUCTION

In previous communications (1) we have shown that cholesterol, when administered subcutaneously in doses of 40 mgm. at intervals of from two to three days, very decidedly accelerates the growth of the Flexner-Jobling carcinoma in rats. This acceleration occurs whether the administration is adjacent to the tumor or distant from it; and not only is the primary growth affected, but the percentage of metastases is very greatly increased.

Our results have been confirmed in a very striking manner by Sweet, Corson-White, and Saxon (2) who, from a tumor which had never previously yielded metastases, obtained, by the administration of cholesterol by mouth, no less than 104 metastases out of 116 inoculations. Nor was this an isolated result, for, employing both normal and restricted diets it was found by these observers that the addition of cholesterol increases the growth of the primary tumor and the incidence of metastases.

Certain comments made by these observers upon their results, as also by Woglom in a recent article (3), have made us realize, however, that our original articles upon this subject must have been deficient in clarity of expression, for our views regarding the mode of action of the cholesterol appear to have

been to some extent, and in important particulars, misunderstood; while Woglom, at all events, has read conclusions into our statements which we never intended to imply.

Thus Sweet, Corson-White, and Saxon, in commenting upon the remarkable effects of administration of cholesterol, make the following statements:

We are nevertheless unable to agree with Robertson and Burnett that cholesterol is a factor of decisive importance. . . . A study of Tables III and V shows that animals placed on the Osborne and Mendel diet have developed, in spite of the cholesterol, the same resistance towards tumor takes that has characterised our work with this diet. It would seem, therefore, that the action of cholesterol must depend not upon its action alone, but upon its action when combined with some unknown element of the food.

These observers appear to believe that there is a lack of harmony between their experimental results and the views concerning the relation of cholesterol to tumor growth which we have expressed in our communications. On the contrary, however, we believe that the results obtained and views expressed by Sweet, Corson-White, and Saxon are in entire harmony both with our experimental findings and our interpretation of these findings.

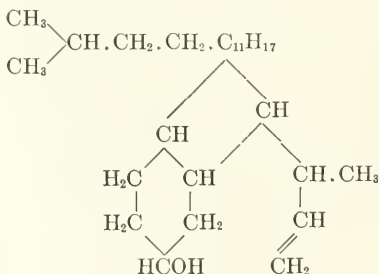
There can be no question but that the action of cholesterol is not attributable to its nutritive value. The nutritive value of the minute quantities of cholesterol employed is a negligible proportion of the total energy-intake of the animals and, moreover, so far as we at present know, cholesterol is not in any large proportion available as a source of energy in the diet. The form of the normal growth-curve of animals (4), as well as the dependence of normal growth upon the presence of minute proportions of certain special factors of negligible calorific value in the diet and upon the normal functioning of the glands of internal secretion, all combine to demonstrate that specific catalysors of growth exist, and we regard and always have regarded cholesterol as constituting one, possibly among many, of these catalysors of growth. Now the Osborne-Mendel diet

employed by Sweet, Corson-White, and Saxon, while supplying a sufficiency of calories and nitrogen, is deficient in lysin, an amino-acid constituent of tissues of which the endogenous metabolism is reducible to zero, but which cannot be synthesized by mammalian tissues. These animals were therefore, in so far as the production of new tissue was concerned, in a condition of starvation, and any new tissue produced, for instance tumor tissue, could only arise by destruction of the tissues of the host. A catalysor cannot accelerate a reaction of which the substrate has already been exhausted, or reinaugurate a reaction in which equilibrium has already been attained. Albeit acids accelerate the hydrolysis of cane sugar, yet when the supply of cane sugar has been exhausted, the further addition of acid will not avail to increase the yield of invert-sugar. We therefore regard the results of Sweet, Corson-White, and Saxon as furnishing substantial support of the view that cholesterol is a catalysor of certain types of growth and particularly of that type which is involved in the growth of carcinomata. Whether we regard cholesterol as a factor of "decisive importance" or not, depends of course upon our definition of what is "decisive." Perhaps, since the raw material or substrate of any chemical reaction is an absolute prerequisite for its occurrence, we might appropriately consider the supply of truly nutritive factors, i.e., calories and the building-stones of tissue, as constituting the only really decisive factors in any type of growth. The underlying idea, both in this remark and in the criticisms of Woglom to which reference has been made, appears to be the mistaken one that we have regarded cholesterol as a cause of tumor growth in the sense that it can initiate tumors. We have, on the contrary, merely expressed the view, which is after all nothing more than a restatement of the facts, that cholesterol is a factor which favors tumor-growth when the conditions precedent to its initiation are present, and we have suggested that the high percentage of cholesterol alleged by Wacker (5) to characterise the subcutaneous fats of aged individuals, may assist materially in determining the increased incidence of carcinoma with advancing age. It must be recollected that the estimation of the

age of incidence of tumors is limited by the possibility of diagnosis. In other words, the necessary conditions precedent to the *initiation* of tumor-growth may be present long before the actual growth of neoplasms renders diagnosis possible. Any condition, therefore, which favours rapid growth in neoplasms once initiated, will play an important part in determining our estimate of the age of incidence.

As a matter of fact we have repeatedly attempted to initiate the growth of neoplasms in normal animals by massive administrations of cholesterol, and we have in every instance failed to obtain any evidence of the development of malignant growths in consequence of such administrations when unaccompanied by the inoculation of tumor-tissue.

The ability of cholesterol to accelerate the growth of carcinomata must undoubtedly depend directly or indirectly upon its chemical structure. We have recently found that another catalysor of growth, namely tethelin, the active principle of the anterior lobe of the pituitary body, is similarly able to cause a very marked acceleration of growth in the Flexner-Jobling carcinoma in rats (6). Now cholesterol and tethelin have this in common: both contain a hydroxy-benzol group. From the investigations of Windaus (7), von Fürth (8) and others, it appears that cholesterol has the formula:



Tethelin has been shown by Robertson (9) to yield inosite, or hexahydroxy-benzol, when split by boiling with barium hydroxid succeeded by sulphuric acid.

The existence of a common radical in these two catalysors of carcinomatous growth necessarily leads to the suspicion that this radical may play an important part in determining their action. It therefore appeared of importance to carry out a series of experiments to determine the effect of modification of this radical in cholesterol upon its ability to accelerate the growth of tumors, and also to determine the effects of a variety of related compounds upon this type of growth. Accordingly the following experiments were undertaken:

II. EXPERIMENTS WITH ACETYL-CHOLESTEROL

Acetyl-cholesterol was prepared from Merck's cholesterol by the method of Raymann (10) and Reinitzer (11) as follows:

Twenty-five grams of cholesterol was boiled with 500 cc. of acetic anhydride for sixteen hours under a reflux condenser. The mixture was then poured into 2 liters of distilled water. The precipitate was washed repeatedly in 2 liter lots of water by suspending it in the water and filtering, until the reaction of the wet precipitate was neutral to litmus. The precipitate was next dried on a water-bath, dissolved in a mixture containing 1 part by volume of alcohol and $1\frac{1}{2}$ parts by volume of ether, filtered, and evaporated until the greater part of the material had crystallized out.

A large yield (25.2 grams) was obtained of acicular white crystals which were slightly yellowish in mass. The melting point was 112°C . (uncorrected). The melting point of acetyl cholesterol is, according to Raymann, 113°C ., and according to Reinitzer, 114°C .

Seventy-two rats were inoculated in the axillary region with the same strain of the Flexner-Jobling carcinoma that we have employed in previous investigations. The "takes," determined after twenty-one days, were 86 per cent. Fifty-six of the animals displaying vigorous growths were then divided without selection into two batches of 28 animals each. The one batch was employed as controls, while the others received 40 mgm. of acetyl-cholesterol suspended in $\frac{N}{10}$ sodium oleate solution

every second or third day, the tumors being measured on the dates of administration. The acetyl-cholesterol suspension was administered subcutaneously on the side of the body opposite the tumor. The following were the results obtained:

TABLE 1

DAYS AFTER IN- OCULATION	AVERAGE DIAMETER OF TUMORS IN MILLIMETERS		AVERAGE DIAMETER OF TUMORS IN PERCENTAGES	
	Controls	Treated	Controls	Treated
21	14.1	13.9	100.0	100.0
23	15.7	14.9	111.3	107.2
25	17.9	16.9	127.0	121.6
28	18.8	18.4	133.3	132.4
30	19.6	19.5	139.0	140.3
32	20.1	19.6	142.6	141.0
35	22.2	21.4	157.4	154.0

It will be seen that there is no evidence whatever of acceleration of the primary tumor due to the administration of acetyl-cholesterol. Nor was any decisive increase of metastasizing tendency noted, for while 24 of the control animals killed sixty-three days after inoculation yielded only 1 case of metastases in the lung, 23 of the treated animals yielded only 2 such cases.

The replacement of the hydroxyl group by an acetyl group therefore robs cholesterol of its power of accelerating the growth of carcinomata. In interpreting this result, it is of importance to recollect that esters of cholesterol are not hydrolysable by lipase, so that unless some special and as yet unknown mechanism exists whereby compounds of this type may be broken down in the tissues, the hydroxyl group is permanently neutralized by esterification.

It is of especial interest to note in this connection that the action of cholesterol in inhibiting the hemolytic action of saponins is also destroyed by replacing the hydroxyl group (12).

We have noted that acetyl-cholesterol differs very markedly from cholesterol in its lack of emulsifiability in sodium oleate solutions. If 4 grams of cholesterol be triturated with a small volume of hot $\frac{N}{10}$ sodium oleate solution, suspended in 100 cc.

of the hot solution, heated to boiling, and allowed to cool, a very stable milky white emulsion of cholesterol is formed (13). Acetyl-cholesterol, when treated in a similar fashion, immediately separates out from the solution, and the mixture therefore has to be thoroughly stirred up before each administration to the animals.

Acetyl-cholesterol, like cholesterol itself, causes large, granu-
lomatous, subcutaneous thickenings at the point of injection.

III. EXPERIMENTS WITH CHOLESTERYL-CHLORIDE

Cholesteryl-chloride was prepared according to the method of Mauthner and Suida (14) as follows:

Fifty grams of cholesterol was rubbed up with 27 grams of phosphorus pentachloride and allowed to stand over night. On the following morning the solid mass was pulverized, and triturated with water, and after the excess of water had been poured off the mixture was heated for some hours on a water-bath. The mixture had then become pasty, and after cooling on ice it was easily crumbled. The mass was now extracted with a large volume of ether and the resultant solution evaporated at room temperature to a small volume; several volumes of alcohol were now added and the mixture allowed to stand overnight. An abundant crop of crystals was deposited and also some yellow waxy globules which were picked out from the rest by hand and rejected. The crystals were dissolved in ether and recrystallized by evaporation at room temperature. The yield was 27 grams of white acicular crystals with a yellowish tinge in mass.

White rats inoculated with the same strain of Flexner-Jobling carcinoma as that used in our previous experiment were sorted out, the small percentage of immune animals rejected, and the vigorously growing tumors separated without selection into two batches. Twenty-one of the animals were retained as controls, and 20 received every second or third day, on the dates on which the tumors were measured, 40 mgm. of cholesteryl-chloride administered subcutaneously on the side opposite the tumor. The following were the results obtained:

TABLE 2
Cholesteryl chloride

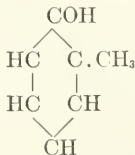
DAYS AFTER IN- OCULATION	AVERAGE DIAMETER OF TUMORS IN MILLIMETERS		AVERAGE DIAMETER OF TUMORS IN PERCENTAGES	
	Controls	Treated	Controls	Treated
21	15.5	14.75	100.0	100.0
23	16.2	15.4	104.5	104.4
25	17.7	17.3	114.2	117.3
28	20.0	19.1	129.0	129.5
30	20.4	20.8	131.6	141.0
32	20.8	22.9	134.2	155.3
35	23.5	22.9	151.6	155.3

No evidence of decisive acceleration such as is obtained with cholesterol is afforded by these results. Neither the controls nor the treated animals exhibited any metastases. We may infer that replacement of the hydroxyl by chlorine, no less than its replacement by an acetyl group, robs cholesterol of its power to accelerate the growth of carcinomata. We also noted that cholesteryl chloride, like acetyl cholesterol, does not form stable emulsions when suspended in hot sodium oleate solution.

The importance of the hydroxyl group in determining the accelerative action of cholesterol upon the growth of carcinomata, which is demonstrated by these results, led us to investigate the action of a number of other hydroxy-benzol derivatives.

IV. EXPERIMENTS WITH META CRESOL

Meta cresol:



contains a hydroxyl group which is directly attached to a methylated benzol ring.

Two batches of 25 animals each were chosen without selection from a number with vigorously growing tumors. One lot was retained as controls, while to the individuals comprising the other batch 1 cc. of a 3.5 per cent solution of meta cresol in distilled water was administered on the dates enumerated in the accompanying table. Nearly all the animals showed symptoms of intoxication consequent upon the administration, and two or three of the smaller animals died from this cause. The following were the results obtained:

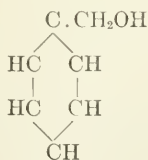
TABLE 3
Meta cresol

DAYS AFTER IN- OCULATION	AVERAGE DIAMETER OF TUMORS IN MILLIMETERS		AVERAGE DIAMETER OF TUMORS IN PERCENTAGES	
	Controls	Treated	Controls	Treated
21	12.4	13.4	100.0	100.0
23	14.3	13.9	115.3	103.7
25	14.3	15.1	115.3	112.7
27	15.7	17.2	126.6	128.4
30	17.6	17.9	141.9	133.6
34	20.4	21.3	164.5	159.0

No evidence of any acceleration of the growth of the tumors consequent upon the administration of meta cresol is afforded by these results.

V. EXPERIMENTS WITH BENZYL ALCOHOL

In benzyl alcohol:



the hydroxyl group is attached to the benzol ring through the intermediation of a methyl group.

Two batches of 25 animals each were employed, chosen without selection from among a number with vigorously growing tumors, one being retained as controls and the other receiving 1 cc. of a 4 per cent (saturated) solution of benzyl alcohol in distilled water every second or third day, administered subcutaneously on the side of the animal remote from the tumor. The following were the results obtained:

TABLE 4
Benzyl alcohol

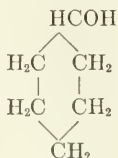
DAYS AFTER IN- OCULATION	AVERAGE DIAMETER OF TUMORS IN MILLIMETERS		AVERAGE DIAMETER OF TUMORS IN PERCENTAGES	
	Controls	Treated	Controls	Treated
21	14.0	13.8	100.0	100.0
23	14.1	15.2	100.7	110.1
25	15.6	15.8	111.4	114.4
28	16.5	17.0	117.9	123.2
30	17.6	18.2	125.7	131.9
32	18.6	18.9	132.9	137.0
35	19.5	20.3	139.3	147.1

While a tendency is displayed in these experiments for the growth of tumors in animals treated with benzyl alcohol to maintain a slight advance over that of the tumors in the controls, we cannot regard this as constituting evidence of a decisive acceleration in any degree comparable with that attainable by the administration of cholesterol. We regard these results as failing to establish any accelerative action of benzyl alcohol upon the growth of carcinoma. Neither the controls nor the treated animals yielded any metastases after fifty days.

VI. EXPERIMENTS WITH HEXAHYDROPHENOL

Benzyl alcohol does not afford a true analogy to the structure of the hydroxy-benzol nucleus in cholesterol, because in benzyl alcohol the hydroxyl is attached to the benzol ring through a methyl group, and, moreover, in cholesterol the benzol group is reduced, i.e., contains hydrogens attached to the ring which

are absent both in benzyl alcohol and in meta cresol. In seeking for a substance more analogous to cholesterol we consulted our colleague Dr. H. C. Biddle, who drew our attention to hexahydrophenol, with a sufficient quantity of which he was also so kind as to furnish us. This substance, which has the formula



is a clear liquid, readily soluble in water.

Two batches of 20 animals each were chosen without selection from a number with vigorously growing tumors, one batch serving as controls and the other receiving subcutaneously 1 cc. of a 4 per cent aqueous solution of hexahydrophenol every second or third day on the side of the animal remote from the tumor. The following were the results obtained:

TABLE 5
Hexahydrophenol

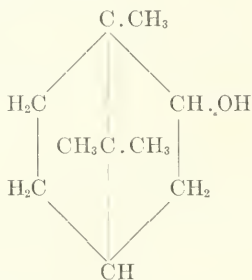
DAYS AFTER IN- OCULATION	AVERAGE DIAMETER OF TUMORS IN MILLIMETERS		AVERAGE DIAMETER OF TUMORS IN PERCENTAGES	
	Controls	Treated	Controls	Treated
21	15.5	16.1	100.0	100.0
23	16.2	16.6	104.5	103.1
25	17.7	19.6	114.2	121.7
28	20.0	21.6	129.0	134.2
30	20.4	23.2	131.6	144.1
32	20.8	24.5	134.2	152.2
35	23.5	25.1	151.6	155.9

Again a slight tendency is displayed for superior growth in the treated animals. The effect is slight and irregular, however, and almost disappears in the last measurement. It is most probably to be accounted for by the initial inequality of the two batches of tumors selected at random. We regard this

experiment as failing to establish any decisive acceleration due to hexahydrophenol. Neither the treated nor the control animals yielded any metastases sixty-six days after inoculation.

VII. EXPERIMENTS WITH BORNEOL

Benzyl alcohol, meta cresol, and hexahydrophenol are all highly soluble substances, while cholesterol is insoluble in water. We therefore sought a substance analogous to cholesterol in the possession of a reduced hydroxybenzol radical and also insoluble in water, arguing that the ready solubility of the substances previously employed might so facilitate elimination that the quantity present in the tissues at any moment would be insufficient to affect tumor growth in any notable manner. We accordingly employed borneol:



with which substance Dr. H. C. Biddle again very kindly supplied us.

Two groups of animals, the one consisting of 25 controls and the other of 20 animals which were subsequently treated, were chosen at random from among a number with vigorously growing tumors, and treated as in previous experiments. The borneol was administered in the form of a 4 per cent suspension in $\frac{N}{10}$ sodium oleate solution, the dosage being 1 cc. of the suspension every second or third day, administered subcutaneously on the side remote from the tumor. The following were the results obtained:

TABLE 6

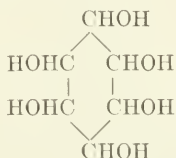
Borneol

DAYS AFTER IN- OCULATION	AVERAGE DIAMETER OF TUMORS IN MILLIMETERS		AVERAGE DIAMETER OF TUMORS IN PERCENTAOES	
	Controls	Treated	Controls	Treated
21	12.4	12.5	100.0	100.0
23	14.3	13.6	115.3	108.8
25	14.3	14.6	115.3	116.8
27	15.7	16.1	126.6	128.8
30	17.6	17.2	141.9	137.6
34	20.4	20.5	164.5	164.0

The results are decisively negative. Shortly after the conclusion of this experiment we lost all our animals through an epidemic, and no record of metastases was therefore obtained.

VIII. EXPERIMENTS WITH INOSITE

This substance, hexahydroxy-benzol,



is of exceptional interest in this connection because it is a product of the hydrolytic cleavage of tethelin, which we have shown to possess a very marked ability to accelerate the growth of carcinomata. Ten grams of this substance, prepared and purified by himself, were very generously supplied to us by Dr. R. J. Anderson, to whom we wish to express our sense of indebtedness.

In these experiments we were forced, owing to the death of our animals, to employ a new strain of the Flexner-Jobling carcinoma, which was very kindly supplied to us by Dr. P. K. Gilman. In its first inoculation into our animals, this strain behaved very differently from the strain to which we have become accus-

tomed. The percentage of takes was as high as in our own strain, i.e., about 90 per cent; but growth during the first three weeks was unusually rapid, so that we commenced administration of the inosite when the tumors were far more advanced in development than in our experiments reported above. Two batches of 22 animals each were chosen at random from among a number with these vigorously growing tumors. One batch was retained as controls, while the individuals comprising the other received 1 cc. of a 4 per cent solution of inosite in water, on the side remote from the tumors, on the dates enumerated in the table. The following were the results obtained:

TABLE 7

Inosite

DAYS AFTER IN- OCULATION	AVERAGE DIAMETER OF TUMORS IN MILLIMETERS		AVERAGE DIAMETER OF TUMORS IN PERCENTAGES	
	Controls	Treated	Controls	Treated
21	20.6	22.9	100	100
23	22.9	23.9	111	104
25	25.0	28.6	121	125
28	27.7	30.9	134	135
30	30.5	33.2	148	145
35	34.8	36.9	169	161

We were unable to regard these results as conclusive, first because of the unusually rapid growth of the tumors in untreated animals, indicating that the velocity of growth, even in the absence of specially administered catalysors, was already at a maximum; and also, because of the accidental disproportion between the initial dimensions of the tumors in the two groups chosen at random for the purpose of comparison. We therefore inoculated a fresh batch of animals from the controls of this experiment and obtained a series of tumors corresponding much more closely with our previous experience of the mode of growth of the Flexner-Jobling carcinoma in rats. From among these animals we selected two batches of twelve animals each, modifying our original random selection so as to render the initial average diameter of tumors in both batches the same. One

batch being retained as controls, the other was treated in the manner outlined above. The following were the results obtained:

TABLE 8

Inosite

DAYS AFTER IN- OCULATION	AVERAGE DIAMETER OF TUMORS IN MILLIMETERS		AVERAGE DIAMETER OF TUMORS IN PERCENTAGES	
	Controls	Treated	Controls	Treated
21	16.8	16.7	100.0	100.0
23	19.3	18.3	115.0	109.7
26	22.4	21.0	133.4	125.7
28	24.1	21.7	142.9	130.0
31	26.1	24.2	155.4	144.9

The results of this experiment are entirely confirmatory of those of the first experiment, and show that inosite administered in the doses specified does not to any appreciable extent accelerate the growth of carcinoma.

IX. SUMMARY OF THE RESULTS

From the results enumerated above we may infer: (1) That the accelerative action of cholesterol upon the growth of carcinoma is dependent upon its hydroxyl radical. (2) That the hydroxy-benzol radical in a variety of other compounds does not produce the characteristic acceleration of the growth of carcinoma that cholesterol does.

Two alternative interpretations of these results offer themselves, between which, at the present moment, we do not feel able definitely to decide. The one is, that the hydroxy-benzol group in cholesterol and tethelin acts as an anchoring group, enabling cholesterol and tethelin to attach themselves to the tumor tissue, while other portions of these molecules are responsible for the stimulation of growth. Against this supposition, in so far as it affects cholesterol, may be urged the fact, ascertained by Bennett (15), that the administration of cholesterol to animals possessing tumors does not lead to any notable increase of the cholesterol content of the tumor-tissue. The

alternative explanation is, that the analogous structures of cholesterol and tethelin, in so far as their common possession of a hydroxy-benzol group is concerned, bear no necessary relation to their analogous actions upon the growth of carcinoma, and that the effect of deprival of the hydroxyl group in preventing the accelerative action of cholesterol upon the growth of carcinomata is attributable to the non-emulsifiable character of derivatives of cholesterol which do not contain a free hydroxyl. This lack of emulsifiability would imply difficulty of distribution throughout the tissue fluids, and this mode of interpreting our results would involve the supposition that the accelerative action of cholesterol is dependent, not upon its concentration within the tumor tissue itself, but in the fluids bathing the exterior and growing parts of the tumor tissue. That the administration of an excess of cholesterol to the animals does lead to a decided increase in the cholesterol-content of the blood, has been recently shown by Luden (16).

CONCLUSION

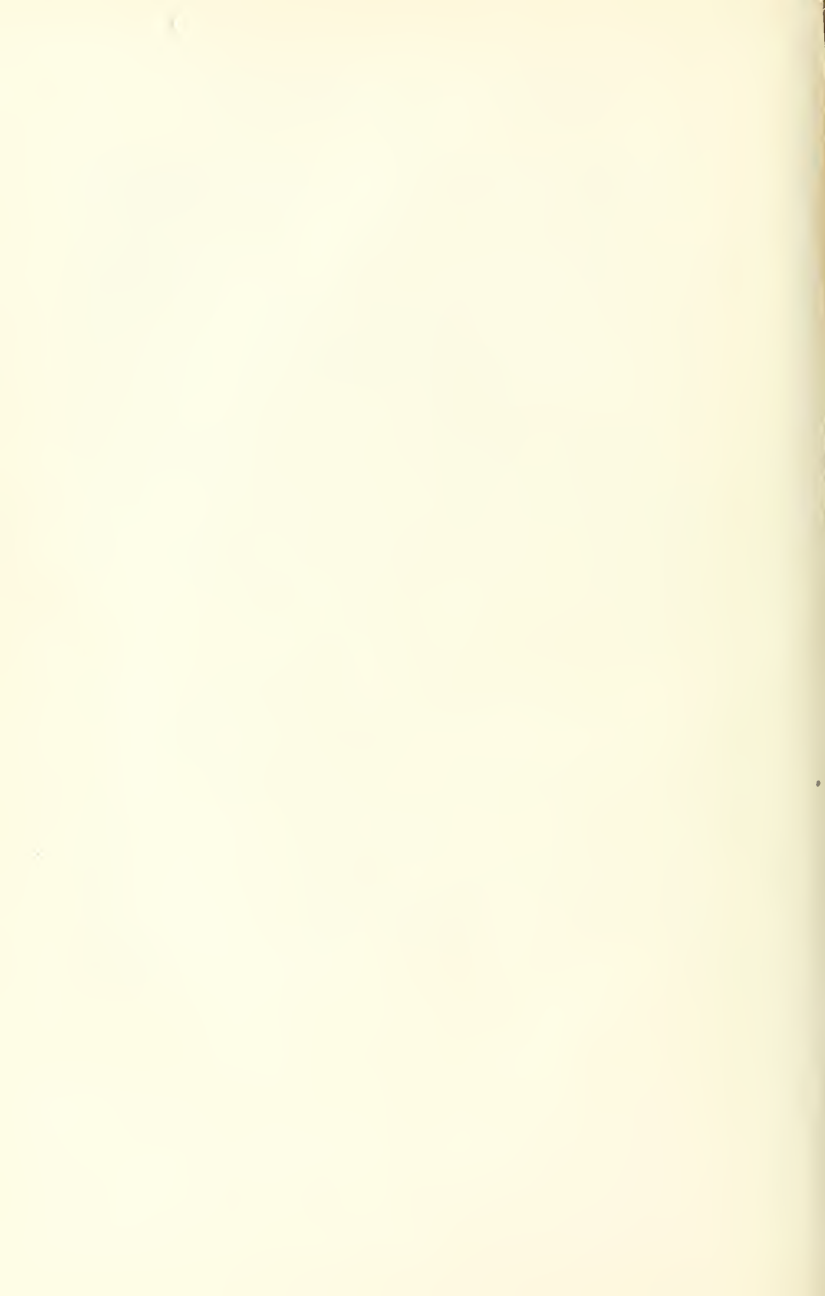
1. The accelerative action of cholesterol upon the growth of carcinoma is dependent upon the hydroxyl radical.

2. Other hydroxy-benzol derivatives, namely meta cresol, benzyl alcohol, hexahydrophenol, borneol, and inosite, do not cause any decisive acceleration of tumor growth.

3. We infer that the accelerative actions of cholesterol and tethelin are not due to their possession of a hydroxy benzol group, but that the accelerative action of cholesterol is in some way rendered possible by its possession of a hydroxyl group. The hydroxyl group may act as an anchoring group, fixing the cholesterol to the tissue, or it may merely facilitate the distribution of cholesterol throughout the tissue-fluids by rendering the cholesterol emulsifiable.

REFERENCES

- (1) ROBERTSON, T. BRAILSFORD AND BURNETT, THEO. C.: Jour. Exper. Med., 1913, xvii, 341.
Proc. Soc. Exper. Biol. and Med., 1913, x, 140 and 143; 1913, xi, 42; 1914, xii, 33.
- (2) SWEET, J. E., CORSON-WHITE, E. P. AND SAXON, G. J.: Jour. Biol. Chem., 1915, xxi, 309.
- (3) WOGLOM, WM. H.: Jour. Exper. Med., 1915, xxii, 766.
- (4) ROBERTSON, T. BRAILSFORD.: Arch. f. Entwicklungsmeehn. d. Organ., 1908, xxv, 581; 1908, xxvi, 108; 1913, xxxvii, 497.
Am. Jour. Physiol., 1915, xxxvii, 1 and 74; 1916, xli, 535.
- (5) WACKER, L.: Ztschr. f. physiol. Chem., 1912, lxxx, 383.
- (6) ROBERTSON, T. BRAILSFORD, AND BURNETT, THEO. C.: Jour. Exper. Med., 1915, xxi, 280; 1916, xxiii, 631.
- (7) WINDAUS, A.: Ber. d. deutsch. chem. Gesellsch., 1912, xlv, 2423.
- (8) VON FURTH, O., and FELSENREISCH, G.: Biochem. Ztschr., 1915, lxi, 416.
- (9) ROBERTSON, T. BRAILSFORD.: Jour. Biol. Chem., 1916, xxiv, 409.
- (10) RAYMANN, B.: Bull. de la Soc. Chim., 1887, xlvii, 898.
- (11) REINITZER, J.: Monatsch. f. Chem., 1888, ix, 167.
- (12) HAUSMANN, W.: Beitr. z. chem. Phys. u. Path., 1905, vi, 567.
- (13) GERALD, E.: Compt. rend. Soc. de biol., 1905, lviii, 348.
- (14) MAUTHNER, F., AND SUIDA, W.: Monatsh. f. Chem., 1894, xv, 85.
- (15) BENNETT, C. B.: Jour. Biol. Chem., 1914, xvii, 13.
- (16) LUDEN, G.: Jour. Biol. Chem., 1916, xxvii, 273.



CASES OF TYPICAL AND ATYPICAL LYMPHOSARCOMA

GEORGE M. MACKENZIE

From the Pathological Laboratory of the Presbyterian Hospital, New York

Received for publication, July 30, 1917

The classical descriptions of Kundrat (1) and Paltauf (2) differentiated lymphosarcoma from the group which in the older literature had all been called malignant lymphomas. The conception of Sternberg, who reviewed the subject in 1903 and added his own observations, is briefly as follows (3): Lymphosarcoma is a tumor formation starting in a group of lymph nodes and spreading thence to neighboring nodes or follicles. It spreads from region to region without ever exhibiting such general lymphoid involvement as leukemia or pseudoleukemia. Sooner or later it invades the capsule and extends into the surrounding tissue. Metastases differ from those of ordinary neoplasms in that the intervening lymphoid tissues are affected. True metastases, which can be explained only through transfer by way of the blood stream, are rare, and usually isolated. Blood-vessels are seldom invaded by lymphosarcoma, being usually only surrounded and narrowed. The commonest sites of origin are the lymph nodes of the neck, mediastinum, mesenteric and retro-peritoneal regions; less frequently the inguinal and axillary nodes. The affected groups form nodular, uneven masses, well limited in the beginning, but later diffusely permeating the surroundings; they are mostly hard, grayish-white, and show a homogeneous cut surface. In general, when a hollow organ is invaded, the growth tends to form a sleeve-like mass about it, usually, however, causing no obstruction, but, on the contrary, tending to widen the lumen. The spleen is rarely attacked and the bone-marrow likewise. Histologically, the tumor is characterized by an irregular reticular framework with lymphoid

cells in the meshes; the architecture of the node is lost; follicles and medulla are no longer differentiated, and the capsule and surrounding tissue are diffusely infiltrated with tumor cells so that the separate lymph nodes can neither be distinguished from one another nor from the surrounding tissue. Often the tumors show an alveolar structure. The cells resemble lymphocytes but are larger, have a more lightly staining nucleus, and a scanty, often almost invisible, non-granular cytoplasm. There is no definite alteration of the blood picture.

The eight cases described by Dr. MacCallum (4) fall quite clearly into this group. Of his cases, none of the three which belonged to the intrathoracic type showed metastases in distant organs. The dissemination of the tumor masses conformed strictly to Kundrat's idea of regional distribution. Indeed, in one of these cases, the tumor had penetrated the heart wall and hung in polypoid masses within the heart without metastasizing by the blood stream. Of the five cases in which the intestinal lesion forms the constant feature, two showed single metastatic nodules in the liver and another had metastases in the liver, thymus, bone-marrow, and kidneys; but these, from the description, seem to have been more like infiltrations, using the framework of the organ as their support, rather than metastatic nodules. The structure of the tumors and the characteristics of the cells, as well as the pathological anatomy in Dr. MacCallum's cases, agree quite closely with Sternberg's description of the Kundrat-Paltauf lymphosarcoma.

The descriptions by Kaufmann (5) and Naegeli (6) agree with those mentioned. The former, however, speaks of two types of lymphosarcoma: the regional and the generalized. The latter he regards as rare, but reports a case of a man, twenty-five years of age, in which a primary lymphosarcoma of the small intestine was associated with metastases in the skin, pericardium, pleura, lung, bone-marrow, and kidneys. Naegeli, too, observes that in the late stages one may find widely distributed nodules.

It is clear, then, that the occasional occurrence of a widespread dissemination of metastases has been well recognized, but

in general these colonies have been described as diffuse infiltrations rather than as distinct circumscribed nodules. The spleen has been considered as almost immune to the tumor.

The four cases included in this report have been taken from the autopsy records of the past five years at the Presbyterian. It would take too long to attempt to give a complete account of each case, but the summaries include the essential features.

Case I. A man of fifty-eight, who six months before admission to the Presbyterian Hospital, in September, 1912, noticed a large lymph node in the left groin. Three weeks later the superficial lymph nodes all over the body were enlarged. The legs, and then the abdomen and scrotum, became swollen. Physical examination showed great enlargement of all the superficial lymph nodes, fluid in the abdomen, enlargement of the liver, and oedema of the external genitals and lower extremities.

Blood count.

Red blood cells.....	3,600,000
Haemoglobin	80 per cent
White blood cells.....	9,600
Polymorphonuclears.....	79
Lymphocytes.....	16
Large mononuclears.....	5
Eosinophiles.....	0

Three weeks later

White blood cells.....	12,000
Polymorphonuclears.....	80 per cent
Small lymphocytes.....	12 per cent
Large lymphocytes.....	6 per cent
Eosinophiles.....	3 per cent

All the lymphatics steadily enlarged and fluid accumulated in the chest. The spleen enlarged so that the edge was felt 6 cm. below the costal border; the edge of the liver was 7.5 cm. below the costal border. A large, firm mass was felt in the pelvis on rectal examination. There is no note in the record of an examination for Bence-Jones protein.

Autopsy. There was a great enlargement of all the superficial lymph nodes. Surrounding the rectum, and nearly filling the pelvis, to the walls of which it was adherent, there was a tumor which extended upward and involved the retroperitoneal lymph nodes, surrounding each ureter, the aorta, and the inferior vena cava. Extend-

ing from the mass about the inferior cava, the tumor had infiltrated the right kidney, diffusing itself gradually out to the cortex. The pericardium was studded with irregular nodules and was adherent to both lungs. The pleural surface of each lung was marked with small nodules and tumor tissue was also found passing into the lung about the bronchi. The mediastinal and bronchial nodes were much enlarged. The liver contained no tumor nodules. The spleen measured 18 by 10 by 7 cm. It showed some small anaemic infarets and on the cut surface innumerable small white foci appearing like enlarged malpighian corpuscles. There was no tumor in the pancreas nor in the left kidney. The oesophagus had a great many very small nodules on the mucous membrane. The stomach and intestine exhibited no evidence of tumor, nor was the lymphoid tissue unusually prominent. The bone-marrow of the femur contained some very small nodules scattered through it.

Microscopical examination. Microscopically the tumor has the same appearance wherever found. The architecture of the lymphoid tissue is entirely lost, the tissue being overwhelmed by tumor cells. The stroma is scanty; the tissue is only moderately vascular. The cells show considerable uniformity in size and shape; they are a little larger than lymphocytes, stain less deeply, and have little or no visible cytoplasm. No tendency toward an alveolar structure is evident. The capsule of the lymph nodes has been invaded, and dense accumulations of cells are found scattered diffusely through the surrounding tissue. In the spleen, the tumor cells have formed follicle-like masses two or three times the usual size of malpighian corpuscles.

Case II. The patient was a man of fifty-five admitted to The Presbyterian Hospital January 4, 1916. He had had a chancre and secondary lesions twenty-three years ago. For seven months before admission to the hospital he had had intermittent sharp darting pains in the back, about the shoulders, and in the groins. Various forms of treatment gave no relief. Physical examination showed a stout plethoric man with slight cyanosis, a little exophthalmos, nystagmus and inequality of the pupils. Heart a little enlarged to the left, systolic murmur at the apex, and occasional extra systoles. There was also a right inguinal hernia. Superficial lymph nodes were not enlarged. Neither liver nor spleen was palpable.

Blood count.

	JANUARY 3	JANUARY 7	JANUARY 8	JANUARY 10	FEBRUARY 1	FEBRUARY 10
White blood cells.....	18,000	25,000	16,800	20,600	14,400	18,200
Polymorphonuclears....	70	73	78	73	70	73
Small lymphocytes....	30	22	17	21	23	11
Large lymphocytes...	0		2	5	2	
Eosinophiles.....	0	2	0	1	0	2
Transitional.....	0	3	2	0		
Haemoglobin.....	90 per cent				5	14

Wassermann. Alcoholic antigen, negative; cholesterin antigen, negative.

Spinal fluid. Normal.

Phthalein. 28 per cent in two hours.

McLean Ambard index. 95.5.

Examination of stool. No blood, pus, ova, or parasites.

Gonococcus complement fixation. Negative.

Pain in various parts of the body persisted, he became paralyzed below the waist, lost control of bladder and rectum, and developed oedema of the legs. By rectal examination a mass was felt above the prostate and hard irregular masses were noted in the left side of the abdomen.

Urine. Specific gravity, 1020 to 1037. Acid. Albumin—very faint trace. Glucose, none. Microscopic, hyaline and granular casts.

Temperature and pulse. Normal till just before death.

Blood pressure. 124/74.

Autopsy. The lymph nodes along the aorta and in the mesentery were greatly enlarged. The grayish tissue by which they were replaced had extended through their capsule and invaded the fat about them, forming a large mass almost completely surrounding the aorta and vena cava from the diaphragm to the pelvis. There were nodules also in the fat about both kidneys. The bronchial lymph nodes were enlarged and composed of tumor tissue. A single node in the lower part of the cervical chain, measuring 1 cm. in diameter, was composed of tumor. The pleural surface of each lung contained nodules, and numerous whitish tumors projected from the liver, the largest measuring 2 cm. in diameter. The spleen weighed 85 grams and seemed to be normal. The dura of the spinal cord, from the 8th dorsal vertebra upward, was infiltrated by a sheet of tumor measuring 1 to 3 mm. in thickness. It had surrounded the roots of the nerves as they passed

out, but did not appear to have exerted pressure on the cord itself, nor were the vertebrae invaded. The other organs showed nothing of interest.

Microscopical examination. Here again the invasive quality and loss of the lymphoid tissue are evident in the sections. In vascularity and in the amount of connective tissue framework, this case is quite similar to the first, but in both the primary tumor and the metastases there is a more marked tendency toward an alveolar arrangement.

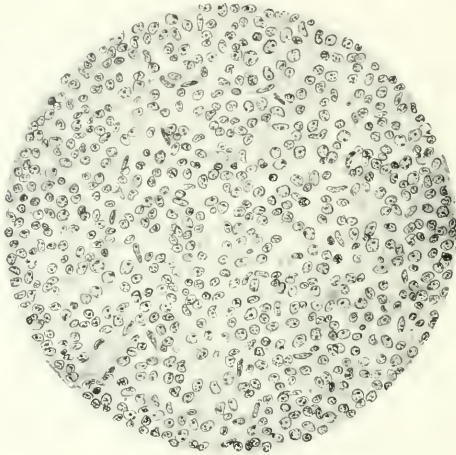


FIG. 1. CASE II

Drawing showing character of tumor cells in the metastatic nodules in pleura and liver. The cells are slightly larger than lymphocytes, are more irregular in shape, and have vesicular nuclei.

Except for their slightly greater size, the cells of the primary growth in the abdominal lymph nodes resemble normal lymphocytes quite closely; they are 8 to 10 micra in diameter, the nucleus is round, deeply stained, and surrounded by only a narrow rim of cytoplasm. In the liver, spinal meninges, and pleural metastases, the cells are larger, more irregular in shape, and have more vesicular nuclei. In the bone marrow there are small discrete foci of tumor cells.

These two cases, it seems, may be classed as belonging to the Kundrat-Paltauf group. In the first, the primary tumor was apparently about the rectum; from here it had extended to practically all the lymphoid tissue in the body and had extensively infiltrated the surrounding tissues. The foci in the spleen and marrow are unusual features but the occasional occurrence of such metastases has been recognized. The primary growth in the second case was evidently in the lymph nodes about the aorta. The invasion of the spinal meninges with the clinical picture dominated by spinal cord symptoms, and the discrete nodules in the liver, were the atypical features here.

Microscopically, both of these cases agree well with the descriptions of Drs. MacCallum, Sternberg, Paltauf, and Kundrat.

The two following cases are more atypical, and hence more difficult to classify.

Case III. The patient was a Russian woman of forty-four, who entered the Presbyterian Hospital August 1, 1916. There was nothing important in the family history or past history.

Seven months before admission to the hospital she noticed enlarged nodes in each inguinal region. Three months later, those in the left axilla became enlarged and subsequently all the other superficial lymph nodes. On physical examination there was found general enlargement of all the superficial nodes, without any tenderness; signs of fluid in the left chest; an enlarged liver, the anterior margin of which extended 8 cm. below the costal border; in the left upper quadrant of the abdomen a very large mass with a notched border taken to be the spleen, and nodular masses in the left lower quadrant also.

Blood count.

Red blood cells...	3,240,000
Haemoglobin	70 per cent
White blood cells	5,300
Polymorphonuclears	85 per cent
Lymphocytes...	13
Eosinophiles...	2

A node was excised from the left axilla and from it an emulsion was made for guinea-pig inoculation. No trace of tuberculosis could be

found in the guinea-pig, however, when it came to autopsy. Examination of the urine for Bence-Jones protein was negative.

Autopsy. At autopsy there were found projecting nodules in the inguinal, axillary, and cervical regions. The lymph nodes about the iliac vessels and the aorta were greatly enlarged, and composed of firm grayish tissue, the largest measuring 6 or 7 cm. in diameter. The capsule of many of the nodes had been invaded, so that in places several were fused together into a single large nodular mass. These masses closely surrounded the aorta, and the vena cava was also embedded in tumor, to which its wall was in places densely adherent. The bronchial and mediastinal nodes were enlarged in the same way and fused into one mass. The psoas muscle on each side, and the lumbar vertebrae, were invaded by direct extension of the tumor from the retroperitoneal nodes.

Lungs. Dense coats of gray tumor tissue were found following the bronchi and blood-vessels into the substance of the lung, and also some button-like nodules on the pleura.

Liver. Weight 1450 grams. It contained numerous projecting nodules of a rather grayish-yellow, translucent appearance; in the region of the cystic duct there was a single large nodule.

Spleen. Weight 1820 grams. It was largely made up of clusters of firm, grayish, more or less discrete nodules of tumor. It showed also large areas of infarction.

Intestines. Normal. No enlargement of the lymphoid structures.

Bladder. There were distinct tumors in the muscular coat.

Uterus, vagina, ovaries, kidneys, adrenals, and aorta. Showed nothing noteworthy.

Bone marrow. The marrow of the lumbar vertebrae was fatty and yellow. In the marrow of the crest of the ileum, a single nodule was found.

Heart, stomach, rectum. Normal.

Microscopical examination. The structure of the tissue and the appearance of the cells is much the same in all the tumor masses. The architecture of the lymph nodes has been entirely lost; there are few blood-vessels and very little stroma, that present being mostly in the form of filaments dividing the tumor cells more or less into groups. The growth has invaded the capsule of the lymph nodes to some extent, but the separation, by the capsule, of the tumor cells within the node from the surrounding tissue, is still distinct. The cells show great variation in size, but average about 12 to 14 micra, though

some are fully three times the size of a lymphocyte; these are often angular and irregular in shape. The well preserved cells have an oval, slightly irregular, or round vesicular nucleus with a sharply marked nucleolus. The cytoplasm is wider than in a lymphocyte and stains a grayish-blue color. Mitotic figures in every stage are abundant. An occasional multinuclear element much larger than the predominating type can also be found after careful searching.

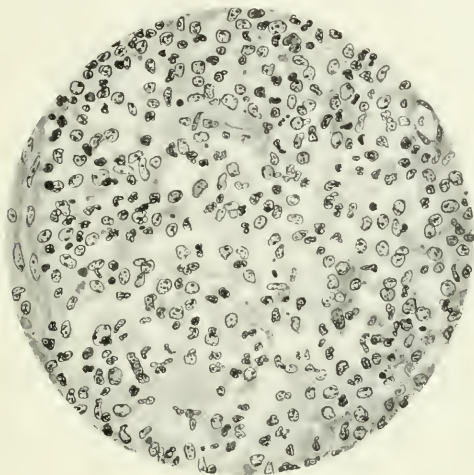


FIG. 2. CASE III

Note the great variation in the size and shape of cells, the presence of fragmented nuclei, the poor vascularization, and the lack of stroma.

Case IV. The patient was an Italian of forty-two, admitted to the Presbyterian Hospital in February, 1914, complaining of swelling of the right leg of about eight months' duration. Physical examination showed a firm mass the size of an egg in the right groin, nodular masses in the right lower quadrant of the abdomen, an enlarged spleen extending 5 cm. below the costal border, no enlargement of the liver, and enlarged nodes in the cervical and axillary groups.

Wassermann. Positive + + +.

Spinal fluid. Negative.

Blood count.

Red blood cells...	5,000,000
Haemoglobin...	85 per cent
White blood cells	6,100 to 14,000
Polymorphonuclears	68 to 78 per cent
Small lymphocytes...	18 to 26 per cent
Large lymphocytes...	2 to 8 per cent
Large mononuclears...	1 to 6 per cent
Eosinophiles...	1 to 4 per cent
Basophiles...	0 to 1 per cent

Urine. A faint trace of albumin and a few hyaline and granular casts.

Blood pressure, pulse, and temperature. Normal.

The superficial lymph nodes steadily enlarged, and became adherent to one another and to the surrounding tissues; great oedema developed in both legs and in the scrotum; the spleen and masses in the abdomen steadily increased in size. The liver was not palpable. A bloody effusion developed in the left chest. Anti-syphilitic treatment had no effect, either upon his general condition or upon the enlarged lymph nodes. Rectal examination disclosed a large mass nearly filling the pelvis. The urine showed no Bence-Jones protein.

Autopsy. In the cervical, axillary, and inguinal regions there were large masses of nodes matted together, none of which, however, was adherent to the skin. The mesenteric and retroperitoneal nodes were greatly enlarged, the largest masses being found just above Poupart's ligament on the right side. In the mediastinum and about the bronchi, the lymph nodes were distinctly enlarged, but less so than in the retroperitoneal chain. The enlarged nodes were generally well encapsulated, although adherent to one another. Nowhere was there a diffuse infiltration of the surrounding tissues. The pleura of the left lung contained large flattened tumor masses; the pleura of the right lung had similar flattened masses but much less extensive. The vessels and bronchi entering the lung were surrounded by coats of whitish tumor, but there were no other nodules in the substance of the lungs. The spleen weighed 2250 grams, the enormous increase in size being due to a great mass of nodules of the same pale gray, firm tumor tissue. A few of the nodules had a yellowish tint and were softer than the others; their average size was about 2 cm. Small masses were also found on

the diaphragm. Heart, aorta, liver, gall bladder, pancreas, adrenals, bladder, and prostate showed nothing noteworthy. The intestines were normal. There was no enlargement of the intestinal lymphoid tissue.

Microscopical examination. The structure of the tumor and the character of the predominating cells are here similar to those in case III, though the nuclei stain somewhat more deeply. In some places, an alveolar arrangement is very definite. The large cells which, in

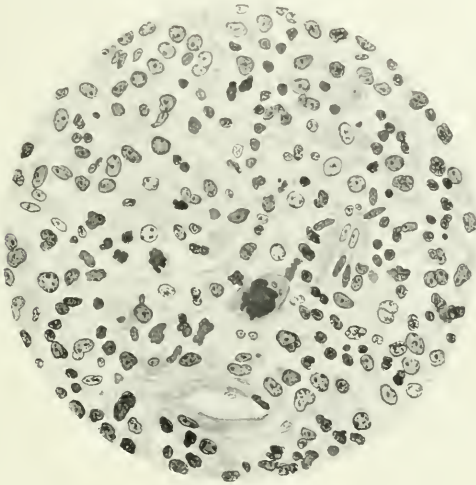


FIG. 3. CASE IV

The cells are very large and vary greatly in shape and size. Extremely large cells with irregularly shaped, deep-staining nuclei are common.

case III, were very scarce, are in this case numerous, almost every low power field having four or five. Some of these have several nuclei closely crowded together, others a single, large, convoluted nucleus. The largest of these elements measure 40 to 50 micra in diameter and have a nucleus in most cases staining very darkly and occupying two-thirds to three-fourths of the cell. The non-granular cytoplasm stains a grayish-pink color and is sharply outlined. These cells resemble

somewhat the megalokaryocytes of bone-marrow, although the nucleus occupies relatively more of the cell and is richer in chromatin. The bone-marrow sections show focal accumulations of cells quite the same as those found in the lymph nodes.

The similarity in these two cases (III and IV), is very striking in many particulars. Clinically, the following points of similarity should be noted:— One patient was forty-two, the other forty-four; in one the disease ran its course in nine months, in the other in fourteen months; in both the first lymphatic involvement noticed was in the groin; in both a marked enlargement of all the superficial lymph nodes followed within a few months; both showed enlargement of the liver and great enlargement of the spleen, as well as nodular masses in the lower abdomen; in neither was there a leukemic blood picture.

In their pathological anatomy the two cases are likewise very similar: the general lymphatic enlargement, the tendency of the enlarged nodes to become fused together into large masses without, however, diffusely invading their surroundings, as was the condition in the first two cases; the great enlargement of the spleen from the presence of discrete tumor nodules closely crowded together, the involvement of bronchi, pulmonary vessels, and pleura in a characteristic manner, the absence of any change in the lymphoid tissue of the intestines, and finally, the presence of discrete foci of tumor cells in the bone marrow without the presence of Bence-Jones protein in the urine. All these features were present in both cases. One of them did, however, show discrete nodular metastases in the liver and the urinary bladder, while the other did not.

The resemblance between the two is further borne out by the histological picture. The structure of the tumor tissue, and the size, shape, and staining affinities of the predominating cells are much the same in each. Case IV has, however, many more of the very large cells and these, moreover, are larger and have more darkly staining nuclei than the large cells in case III.

When we try to place these two cases in any of the recognized groups of primary enlargement of the lymphoid tissues,

difficulties are encountered at once. From the Kundrat lymphosarcoma they are distinguished by the following characteristics:

1. The early involvement of the lymph nodes throughout the body, in a manner which does not suggest a regional extension.
2. The formation, in great numbers, of discrete nodular metastases in the liver (case III) and spleen (both cases).
3. The much less marked tendency to grow out into the surrounding tissues, but rather to form large masses of nodes, adherent to one another.
4. The very different histological picture: -the greater average size of the cells and the variation in their size and shape, the more vesicular character of the nuclei, and the presence of very large cells which in one case have an appearance suggesting megalokaryocytes.

From pseudoleukemia or aleucoeythaemic lymphatic leukemia these cases are readily differentiated by the type of splenic enlargement, the nodular metastases, and the different histological picture, these cases having cells bearing no resemblance to the lymphocyte.

From the leucosarcoma of Sternberg, assuming that there may be an aleukemic stage or form of this condition, the cases I have described may be differentiated:

1. By the fact that in leucosarcoma the splenic enlargement is due to a diffuse infiltration of the whole spleen by the abnormal cells, and not to the presence of clusters of discrete nodules.
2. The liver is affected in leucosarcoma in the same way as in leukemia, that is, by infiltrations.
3. By the absence in leucosarcoma of the very large cells found in the two cases I have described.

It is hardly necessary to point out that the histological picture alone of Hodgkin's disease is sufficiently characteristic to make it unnecessary to indicate other points of differentiation.

SUMMARY

In conclusion, then, the four cases included in this preliminary report fall into two groups. The first two have enough in

common with the Kundrat-Paltauf lymphosarcoma to be classed as such. The other two cases differ so essentially from lymphosarcoma, pseudoleukemia, and leucosarcoma that to place them in any of these groups seems unjustifiable.

REFERENCES

- (1) KUNDRAT: *Wien. klin. Wchnschr.*, 1893, vi, 211.
- (2) PALTAUF: *Ergebn. d. allg. Path.*, 1896, 3¹, 652.
- (3) STERNBERG: *Ergebn. d. allg. Path.*, 1903, 9², Abt. 360.
- (4) MACCALLUM: *Tr. Assn. Am. Phys.*, 1907.
- (5) KAUFMANN: *Pathologische Anatomie*, Berlin, 1911.
- (6) NAEGLI: *Leukaemie und Pseudoleukaemie*, Leipzig, 1913, p. 186.

RESEARCHES ON THE CANCEROUS DISEASES IN NORWAY

AN ABSTRACT¹

F. G. GADE

Secretary to the Norwegian Committee for Cancer Research

Received for publication, July 30, 1917

INTRODUCTION

The Kingdom of Norway occupies the northern and western part of the Scandinavian Peninsula, with a coastline of 1700 miles fronting the Skagerack, the North Sea, and the Atlantic and the Arctic Oceans.

The total area is 124,495 square miles (thus slightly exceeding that of Great Britain and Ireland). The configuration of the country is very extended, making the distance from its southern point, Cape Lindesnes at 58° N.L., to the North Cape at 71° N.L., about 13 degrees of latitude, or 1000 miles or more as the crow flies.

The country is mountainous throughout its whole extent; the greatest elevations are to be found mainly near the western coast, which is protected by innumerable islands and indented by deep, ramified fjords that reach far into the mountainous inland. This is also divided by mountain chains into a great number of valleys, in the southern part of the country mostly converging and opening towards the south-eastern low-lands, in the vicinity of Christiania, the capital.

Only about 4 per cent of the soil is cultivated, 7 per cent being pasturage, 21 per cent forests, and 69 per cent naked mountains, lakes, glaciers, etc.

¹ Undersøkelser over Krafpsygdommene i Norge (Videnskapselskapets Skrifter. I. Mat-Naturv. Klasse 1916. No. 7), Kristiania.

The population amounts to 2,400,000 inhabitants, irregularly and with greatly varying density scattered over the vast area. Of this population, 71.1 per cent live in the country, and only 28.9 per cent in towns, Norway thus showing a very low percentage of urban population.

Ethnographically, the Norwegian people is very homogeneous; it belongs nearly exclusively to the northern (Scandinavian) branch of the Indo-germanic race. However, chiefly in the far north, there live about 10,000 Finlanders and 20,000 Lapps, both belonging to the Ugrian race.

The Norwegians are generally fair-haired and blue-eyed; the shape of the skull is dolicho- or mesocephalic, and the stature rather tall, Norwegian recruits (of 23 years) averaging a little more than 171 cm. in height. Thus they are perhaps the tallest men in Europe.

On the southwestern coast there is a distinct strain of a brachycephalic fair-haired and tall population.

The average of longevity is very high, the average length of life being 54.8 years for men, 57.7 for women, next to Denmark the highest in Europe. This is, perhaps, mostly due to the low infant mortality.²

During the period included in the following researches, Norway has been divided into 158 districts for its medical administration.

The material on which these researches are based is: (1) The official mortality reports for the years 1902-1911, and (2) the material collected by the Norwegian Committee for Cancer Research, 1908-1912.

I. MORTALITY STATISTICS, 1902-1911

1. *The increase in the mortality from cancer*

The official medical reports of the last half century show a continuous rise in the number of deaths from malignant tumors, from 115 deaths in 1854 to 2182 in 1904, i.e., a proportion of 1 to 18.9.

² The average length of life in the United States is 48.3 years for men, 51.9 for women.

But this appalling increase is, of course, only apparent. During the same 50 years the population has grown in the proportion of 1 to 1.6, and the knowledge of the causes of deaths in the proportion of 1 to 7.2 (from 11.9 per cent to 86.3 per cent of all cases).

YEAR	POPULATION MILLIONS	REPORTED DEATHS FROM CANCER	PERCENTAGE OF KNOWN CAUSES OF DEATH	NUMBER OF DOCTORS IN NORWAY
1854	1.447	115	11.9	315
1864	1.658	296	38.4	354
1874	1.774	568	42.9	463
1884	1.922	1006	52.7	610
1894	2.030	1494	68.3	848
1904	2.274	2182	86.3	1138
Increase.....	1: 1.6	1: 18.9	1: 7.2	1: 3.6

The real increase in the mortality due to malignant tumors during these 50 years, 1854-1905, will consequently not exceed the ratio of 1 to 1.66. And even this figure may probably be somewhat reduced, considering that a constantly increasing number of deaths has been recognized as due to malignant tumors. Still it can not be denied that the figures given tend to show a certain increase in the number of deaths in Norway referable to cancerous diseases, from the middle of the last century up to the present time.

2. Present mortality

During the years 1902-1911, the total mortality from all malignant tumors (carcinoma and sarcoma) in Norway amounted to 22,111 cases, varying slightly from year to year. (In 1914, the cancer mortality was reported to be 2484 cases).

This makes an average annual mortality of 9.5 to 10,000 living, about 7 per cent of the total mortality from all causes.

But during the period in question, the percentage of known causes of death was 87.4, 12.6 per cent of all deaths not being established by the health officers. The above reported number of deaths by malignant tumors may consequently be augmented by 12.6 per cent, in order that the exact figure may be arrived at. If this be conceded, *the mortality from malignant*

tumors in Norway during the years 1902-1911 may be estimated at 10.7 per 10,000, the "cancer figure."

The cancer mortality—cancer figure—calculated in this way varies not inconsiderably in the twenty different counties (*amts*) through the country, this variation ranging from 8.1, in Finnmarken (north) to 13.8 in Jarlsberg and Larvik (south), this latter having a very much denser population. As a rule, the counties are of considerable extent, varying greatly within their own boundaries as to soil and living conditions, and consequently, also, as to the conditions favorable to the development of disease. A truer idea can be derived by examining the 158 medical districts, each of them geographically and ethnographically more homogeneous and well-defined.

The cancer mortality in these districts varies considerably more than in the counties, the cancer figures in the districts ranging from 23.2 to 1.8 per 10,000. It must be remembered, however, that the percentage of unknown causes of death varies highly in the different districts, ranging from 0 per cent to 50 per cent, and reaching in one district even as high as 65.2 per cent; this may in some cases make the results obtained less reliable.

The results of these detailed investigations, as shown by the accompanying map, prove that the regions about the Trondhjemsfjord (from 63° to 65° north latitude) represent the greatest continuous cancer-area in Norway.

Among the highly cancerous districts must also be mentioned the well populated districts to the east and south of Lake Mjøesen, as well as the whole of the densely populated county of Jarlsberg and Larvik.

Least affected by malignant tumors are the northernmost counties of the country (Nordland 9.2, Tromsøe 8.8, Finnmarken 8.1), as well as some districts along the western coast and the fjords there (Hardanger only 7.0), and a few stray districts in the southern part.

The urban population of Norway represents, as has been said above, a rather small part of the entire population, smaller perhaps than in any other country in Europe. The cancer mortality is seen to be distributed between cities and rural districts

in about the same proportion, showing an excess of only 1.6 per cent against the urban population.

	POPULATION	CANCER MORTALITY
	<i>per cent</i>	<i>per cent</i>
Cities.....	28.9	30.5
Rural districts.....	71.1	69.5

It must be remembered, however, that the two largest cities, Christiania and Bergen, representing together about 42 per cent of the whole city population, show a lower cancer mortality than the average for the whole country; and that a considerable number of cities of 10,000 and more inhabitants also possess a cancer mortality not exceeding the cancer figure for all Norway, 10.7.

On the other hand, a number of small towns along the Skagerak coast (south) show very high cancer figures (from 14.0 to 25.8), perhaps because these places are largely inhabited by old, retired, sailors.

As a whole, though, it seems that town life in Norway does not especially favor the development of cancer; perhaps rather the contrary is the case.

3. *Carcinoma and sarcoma*

Of the whole number of deaths from malignant tumors (22,111), sarcoma accounts for 1185 (5.36 per cent), whereof 619 (52.2 per cent) are of males and 566 (47.8 per cent) of females.

There has been a slight variation in the frequency of sarcoma between single years within the period, from 4.35 per cent (1908) to 6.14 per cent (1905), though without any regular movement being discoverable. A similar variation can be noted among the counties during the whole period, from 3.34 per cent (Jarlsberg-Larvik) to 6.51 per cent (Nordland).

The highest percentage of sarcoma has, as a rule, been reported from the northern and western coasts, the smallest from the inland districts.

4. Distribution of malignant tumors as to sex and organs

The ratio as to sex will be seen in the following summary (1902-1911):

	MALES	FEMALES	TOTAL
Carcinoma	10,092 (48.5%)	10,724 (51.5%)	20,816
Sarcoma.....	621 (52.2%)	569 (47.8%)	1,190
Total.....	10,713 (48.7%)	11,293 (51.3%)	22,006
Population 1910.	1,155,673 (48.3%)	1,236,109 (51.7%)	2,391,282
Individuals above 35 years of age.....	353,243 (45.6%)	421,510 (54.4%)	774,753

This makes it apparent that *malignant tumors in Norway are very equally distributed between the sexes*, all ages being taken into account.

With regard to ages above 35 years, there is some preponderance of carcinoma among males, and a somewhat greater one in the case of sarcoma.

The Norwegian mortality statistics up to 1910 have specified very inadequately the localization of carcinoma. Only the stomach, liver, genital organs (external and internal), and mamma have been specially mentioned. The following summary gives the figures for the years 1902-1911:

	MALES	FEMALES	TOTAL
Carcinoma			
Stomach.....	7,037	5,712	12,749
Liver.....	751	755	1,506
Sexual organs.....	125	1,343	1,468
Mamma.....	4	838	842
Other organs and unspecified.	2,175	2,076	4,251
	10,092	10,724	20,816
Sarcoma			
Osseous system.....	258	197	455
Other organs and unspecified	363	372	735
	621	569	1,190
Total.....	10,713	11,293	22,006

The *predominance of gastric carcinoma* is very conspicuous—61.2 per cent of all carcinomas; furthermore, a majority of the liver carcinomas may doubtless also be added to this group. Carcinoma of the stomach is more prevalent among males, who furnish 55.2 per cent, while females contribute but 44.8 per cent. This excess among males is, in reality, still more prominent than it seems, for the number of males and females in Norway above 35 years has been found to be relatively 44.6 per cent and 54.4 per cent (census of 1910). Consequently, where 100 men above 35 years of age die from cancer of the stomach, only 68 women succumb to this disease.

For the year 1911, the diagnoses have been somewhat more carefully specified, accounting thus for carcinoma of the various internal sexual organs. One hundred and thirty-six cases of uterine cancer having been reported for this year alone (9.2 per cent of all carcinomas of the female genitals during the whole decade), it may be concluded that carcinoma of this organ will account for 80–90 per cent of all deaths from genital cancer in this country.

5. Age

The cancer mortality in Norway exhibits the same well known relation to age as everywhere else; only a very few cases occur in individuals below 20 years of age, after which there is a gradual increase in frequency through every decennium up to the higher age periods until the absolute maximum of deaths is reached at 60 to 70 years; thereafter a slow decline takes place. The relative maximum of deaths, however, is found in the group of 70 to 80 years, after which a decrease sets in.

For carcinoma alone the figures for the period 1902–1911 are given in the table below.

Whether this decline after the 70 to 80 period is real, or only apparent, is difficult to decide. But a great number of deaths from cancer among aged people is doubtlessly hidden under the diagnosis “senile debility,” a diagnosis which plays a rather too prominent part in our mortality statistics, being second only, indeed, to that of tuberculosis.

AGE	NUMBER OF REPORTED DEATHS	NUMBER OF DEATHS PER 100,000 LIVING
20-30	108	3.23
30-40	586	21.25
40-50	2,067	91.79
50-60	4,498	231.65
60-70	6,183	443.03
70-80	5,610	680.11
80-90	1,790	549.90
over 90	88	289.00

The reports mention 21 deaths (0.95 *pro mille*) from carcinoma below the age of 20 years, but as it is not stated whether the diagnoses were based upon the findings at operation or post-mortem examination, or whether they were made from microscopical preparations, these cases can be accepted only with reservation.

6. *The relation between the number of aged people and the frequency of cancer*

The undeniable connection between advanced age and a predisposition toward cancer, gave rise to the idea that the wide variations in the number of cancer deaths in different districts might be brought into connection with similar variations in the numbers of aged persons resident in them. There might prove to be, in other words, a certain parallelism between the percentage of cancer deaths (the cancer-figure) and that of aged persons (the age-figure) in the various districts.

To test this theory, a calculation was made (national census of 1910) of the relative number of individuals above 35 years of age in the individual districts, as well as in the whole country.

It was found that 32.4 per cent of the entire population of Norway were of the said age, but that the percentage, the age-figure, varied for the districts from 42.5 (most aged population) down to 20.5 (young population), the variation of the age-figure in the towns being from 45.2 to 21.0.

A high age-figure is mostly found in the mountain regions, in the inner parts of the fjords, and in the agricultural districts;

also in a number of small towns along the southern coast (where old sailors are settled), the same towns in which the cancer-figure was shown to be high.

A low age-figure is very marked in all the northern parts of the country (in the plurality of the districts north of 65° N.L.), on some parts of the western coast, and in the great industrial centers.

To a certain extent, a coincidence of low or high age- and cancer-figures could certainly be noted. In the northern districts both figures are mostly low, while in the southern towns both are high; and a considerable number of districts and towns possessing age-figures of medium height also exhibited cancer-figures upon much the same level. But the expected parallelism between the two can not be demonstrated a ruling law; at both extremities of the scale the relations between the age-figures and cancer-figures may assume a rather paradoxical aspect, districts and towns with a low age-figure (young population) showing a very high cancer-mortality, and those with a high age-figure (aged population) being nearly exempt from cancer.

Consequently a direct connection between the number of individuals above the age of 35 years and the number of deaths from cancer in a given district can not be demonstrated.

II. THE MATERIAL COLLECTED BY THE NORWEGIAN COMMITTEE FOR CANCER RESEARCH, 1908-1912

The mortality statistics give at present the only reliable information as to the general frequency and the geographical distribution of cancer in Norway. But these statistics contain very little information, or none at all, regarding the distribution of the disease among different organs, among the various trades and occupations, and about the families, dwellings, etc. of the patients. At this point we must recur to the material gathered by the Norwegian Cancer Committee, though this being entirely dependent upon voluntary contributions from practitioners all over the country, has been forthcoming rather irregularly, and

does not allow of any immediate comparison with the official mortality statistics. This material, consisting of 4219 cases (living and dead), has some peculiarities.

Thus it shows a considerably greater surplus of females (57.5 per cent against males 42.5 per cent) than the official mortality statistics do; this is perhaps because of the frequently accessible localization of cancer in women (uterus and mamma, amounting to nearly 26 per cent of the whole collected material), which makes for easy diagnosis. Hence they are more often reported than cases of cancer affecting the stomach, say, which is the most frequent site in men.

1. Distribution of malignant tumors according to sex and to the organ involved

(The diagnoses concern only the primary localization).

	MALES	FEMALES	TOTAL	PERCENTAGE OF ALL CASES RE- PORTED
A. Carcinoma				
Cutis.....	131 (60.0%) +	88 (40.0%) =	219	5.2%
Labium.....	289 (88.7%) +	37 (11.3%) =	326	7.8%
Cavitas oris.....	95 (56.6%) +	76 (43.4%) =	171	4.1%
Oesophagus.....	56 (55.4%) +	45 (44.6%) =	101	2.4%
Ventriculus.....	747 (57.0%) +	563 (43.0%) =	1310	31.4%
Intestinum.....	142 (46.8%) +	161 (53.2%) =	303	7.2%
Hepar et cyst. fell.....	27 (37.0%) +	46 (63.0%) =	73 ¹	1.8%
Pancreas.....	19 (59.4%) +	13 (40.6%) =	32	0.8%
Tractus respir.....	13 (55.0%) +	11 (45.0%) =	24	0.6%
Tractus urin.....	22 (50.0%) +	21 (50.0%) =	42	1.0%
Genit. externa.....	22 (35.0%) +	41 (65.0%) =	63	1.5%
Genit. interna.....	29 (5.5%) +	499 (94.5%) =	528	12.6%
Mamma.....	5 (0.9%) +	539 (99.1%) =	544	13.2%
Organa div.....	13 (27.0%) +	34 (73.0%) =	47	1.1%
	1610 (42.5%) +	2174 (57.5%) =	3783	90.7%
B. Sarcoma.....				
	165 (42.3%) +	225 (57.7%) =	390	9.3%
	1775 (42.5%) +	2399 (57.5%) =	4173	

A. Carcinoma. Carcinoma of the stomach, with 31.4 per cent of all cases, occupies first place also in these reports, even though it is only a little more than half as frequent as stated in the mortality statistics (61 per cent). It is somewhat surprising to find the rather rare primary cancer of the liver reported is as many as 55 cases (+ 18 cases of *c. cyst. felleae*), and it may be safely assumed that the great majority of these were primarily cancer of the stomach. In only 6 cases has the diagnosis of primary cancer of the liver been based upon laparotomy or autopsy, and microscopical examination. The frequency of cancer of the liver should accordingly not exceed 0.14 per cent.

In second place comes cancer of the internal and external genitals, and of the breast, comprising together 27.3 per cent of all reported cases, and showing in both groups an overwhelming majority of the cases in women.

The carcinoma of the external genitals ought, to a great extent, to be classed as cancer of the skin.

Cancer of the lip is much more frequent in males (88.7 per cent) than in females (11.3 per cent), the lower lip being the seat of the tumor in 95.7 per cent, the upper lip in 4.3 per cent only of the cases. This seems to strengthen the old suspicion against the tobacco pipe, and particularly the clay pipe, as the causal factor, the more so as lip-cancer in Norway is geographically almost exclusively limited to certain remote valleys and forest and fishing districts on the western coast, where the clay-pipe is still, perhaps, a favourite. On the other hand, only a very few cases of lip cancer have been reported as originating in individuals born and living in towns (mostly from Stavanger).

2. Distribution of carcinoma as to localization, sex, and age

Only 3785 out of the 4219 reports give the information necessary to a proper estimate.

More than 26 per cent of all these cases occur in the years from 60 to 69, and nearly 25 per cent in the 50 to 59 year group; consequently more than half of all cases are found in the 50 to 70 year group.

	UN- DER 20	20-29		30-39		40-49		50-59		60-69		70-79		80-89		90 AND MORE		AGE NOT RE- PORTED			
		M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.				
1. Cutis.....	2	2	1	6	4	13	15	26	21	34	24	33	20	7	7			2			
2. Labium.....		3	1	8	1	27	4	69	7	94	9	60	10	25	5			3			
3. Cavitas oris...				3	2	12	15	17	21	36	22	20	13	5	3	1		1			
4. Oesophagus....				4	4	6	3	15	8	14	14	14	11	2	4			1			
5. Ventriculus...		4	4	31	31	110	83	203	139	234	167	130	100	25	21	1	5	9			
6. Tract.intestin.		3	2	4	6	14	23	32	37	53	50	23	28	10	10	2	1	4			
7. Hepar.....				3	2		5	8	15	12	11	3	6	1	7						
8. Pancreas.....		1				4		6	4	5	4	3	4		1						
9. Tract. resp....				1	1	3		2	8	4	1	3	1								
10. Renl. Tract. urin.....				1	1	3	6	5	6	3	5	4	3	2	1			1			
12. Genitalia ext..				2	4	1	8	6	9	4	10	9	8		2						
12a. Genit. int. viril.....				3		1		6		5		10		1				2			
12b. Genit. int. femin.....		4		27		66		147		137		75		28		2		14			
13. Mamma.....				14		75	1	181		117	4	91		36		13	1	11			
14. Diverse gland.		1		1	1	1	3	8	1	7	4	4	0	2							
15. Not localized..						2	3		4	2	2							1			
		2	5	13	51	67	200	203	497	396	540	508	489	312	270	78	76	4	7	20	45
		7		64		267		700		936		997		582		154		11		65	
m. 1603 + f. 2182																					
3785																					

The decade 60 to 69 years shows in both sexes the greatest number of carcinomas of the skin, lips, mouth, oesophagus, stomach and intestine, external genitals, and internal genitals in males.

On the other hand, the maximum for carcinoma of the internal female genitals, as well as of the breast, occurs as early as the 40 to 49 decade, and the frequency-diagram shows that cancer of the uterus and of the breast follow each other very closely throughout all ages, both reaching their climax during the forties. Cancer of the stomach in women, on the contrary, reaches its highest figure during the sixties.

This early appearance of cancer in the female generative organs may, perhaps, be connected with the earlier biological senility of these structures.

3. Carcinoma in the young

Carcinoma in persons less than 30 years of age has been reported in 71 cases (1.68 per cent)—15 males and 56 females. In young men, we find the tumor located in the skin (4 cases), stomach (4), lips (3), intestine (3), and pancreas (1). In young women, the localisation is most frequently in the internal genital organs (uterus, 18, adnexa 3, ovarium 12, = 33), and in the breast (14).

Up to the age of 25 years, only 24 cases are reported, as the following table shows:

	AGE																TOTAL					
	14		15		18		19		20		21		22		23				24		25	
	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
Ovarium.....		1				2		1						1				1		2		8
Uterus.....													1			1				2		4
Mamma.....									1		1									2		4
Cutis.....			1		1														1		2	1
Parotis.....											1											1
Ventriculus...												1						1			2	
S. Roman.....								1												1		
Ren											1											1
		1	1		1	2		1	1	1		3	1	2		1	1	1		7	5	19
		1		1		3		1		2		3		3		1		2		7		24

It will be seen that only 8 cases are reported in patients under 20 years of age (3 males, 5 females); of these, two young men (of 15 and 16 years) suffered from carcinoma cutis nasi, and one from carcinoma S. Romani. Of the women, four had carcinoma ovarii and one carcinoma mammæ. These eight cases had all been verified by laparotomy on postmortem section, and microscopical examination.

B. Sarcoma. As seen in the table on page 116, the sarcomas

play a considerably greater part in these reports than in the mortality statistics, amounting to 390 cases out of 4219, or to 9.3 per cent as against 5.36 per cent. They show a preponderance in females (57.7 per cent), the figure for males being 42.3 per cent.

As usual, the sarcomas occur more uniformly within all age-groups, 23 cases (6 per cent) occurring in individuals under 10 years of age, though the fifties, with 64 cases (16.2 per cent), show the maximum incidence.

The sarcomas have in most cases developed from the skin and connective tissues (42 per cent), or from the osseous system (27.6 per cent). From the muscular system 9 sarcomas are reported, of which no less than 8 arose from *m. rectus abdominis*.

4. *Multiple malignant tumors*

In this category are included only those cases in which two or more malignant tumors have developed in the same individual, either simultaneously or successively, but both (or all) being of such a nature or appearing under such conditions that no connection could be found between them; in other words, none were metastases or recurrences.

Among the 4219 reported cases, 32 (0.76 per cent) multiple tumors were to be found, 31 double and 1 triple tumor. The combinations in which they occurred were as follows:

Carcinoma + carcinoma.....	25
Carcinoma + sarcoma.....	6
Sarcoma + sarcoma.....	1

The triple carcinoma had its localization in the stomach, the coecum, and the flexura coli d.

In 15 of the double cases, the first tumor observed developed from the squamous epithelium of the skin (9) and the lips (6). In 12 cases (6 from skin, 6 from lips) the second tumor also developed from the squamous epithelium, but only once was it so localized that a suspicion of direct inoculation might be justified (corresponding places on both lips); in this case, however, the first tumor had been extirpated 16 years before the second one appeared, and there had been no recurrence.

On the whole the length of time intervening between the first and the second of these skin and lip carcinomas was rather long, running from 3 to 25 years; in only two cases did the two growths appear within the space of two years.

Of the 5 cases of multiple tumors developing from the stomach and alimentary canal, 4 seem to have been simultaneous; in the remaining case there was an interval of only one year.

The 5 cases with two mammary carcinomata also exhibited both tumors almost simultaneously in both breasts, or within not more than 3 to 4 years.

5. The geographical distribution of the various cancer sites

This can not be sufficiently shown through these reports, as they have been forthcoming with too great an irregularity. It is nevertheless well known that cancer is found at widely different sites in different parts of the country. Thus Dr. Munch Soegaard and Dr. Garman Andersen found years ago that carcinoma uteri is exceedingly rare in the district of Hardanger, and it has already been mentioned that cancer of the lip is to a great extent limited to the remote valleys, to the highlands, and to some few districts on the south western coast.

6. The distribution of carcinoma of various organs according to social position and occupation

The social position and occupation, which have been reported in 2554 cases under about 80 different classifications, have here been collected into 9 groups, each containing from 1034 to 55 persons: (1) Farmers (1034); (2) factory employes (538); (3) skilled laborers (285); (4) female servants (205); (5) sailors and fishermen (176); (6) business men (129); (7) minor government and municipal officials (67); (8) teachers, telegraphers, etc. (55); (9) officials, officers, artists, university graduates (65).

The stomach, skin and lips, uterus, and mamma, have been considered according to the relative frequency with which they are involved in each of these 9 groups.

The percentage of cancer of the stomach to all cancers is:

	TOTAL	MALES	FEMALES
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Minor officials.....	62.7	88.6	34.4
Skilled laborers.....	57.2	83.3	30.8
Laborers.....	51.1	70.3	32.9
Farmers.....	48.5	56.3	39.0
Sailors and fishermen.....	43.7	76.2	25.7
Business men.....	38.8	81.6	20.8
Female servants.....	38.5		38.5
University men, artists.....	33.9	77.7	17.0
Teachers, etc.....	32.7	68.7	17.9

Carcinoma [cutis et] labii shows quite different figures, as follows:

	TOTAL	MALES	FEMALES
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Farmers.....	30.0	43.7	13.0
Laborers.....	17.8	29.7	7.2
Teachers.....	16.4	21.3	10.3
Sailors, fishermen.....	15.3	23.8	8.2
Skilled laborers.....	14.7	16.2	13.3
Minor officials.....	11.9	11.4	12.5
Officials, artists.....	9.1	22.3	4.3
Business men.....	7.0	17.4	2.2
Female servants.....	4.0		4.0

With the carcinomata peculiar to women we find that cancer of the uterus exhibits the following frequency:

	CALCULATED WITH REGARD TO WOMEN ONLY	FOR THE WHOLE GROUP
	<i>per cent</i>	<i>per cent</i>
Laborers.....	35.6	18.4
Sailors and fishermen.....	34.8	21.6
Minor officials.....	28.1	13.4
Female servants.....	27.3	
Skilled laborers.....	27.2	13.7
Business men.....	25.3	17.8
Farmers.....	17.1	7.5
Officials, artists.....	17.0	12.3
Teachers.....	10.3	7.3

Cancer mammæ showed on the other hand:

	WOMEN ONLY	FOR THE WHOLE GROUP
	<i>per cent</i>	<i>per cent</i>
Officials, artists	61.7	44.6
Teachers.....	61.5	43.6
Business men.....	51.7	36.4
Farmers.....	31.4	14.0
Sailors and fishermen.....	31.3	19.3
Female servants.....	30.2	
Skilled laborers.....	28.7	14.4
Minor officials.....	25.0	11.9
Laborers.....	24.3	12.6

The figures are much too small to allow of basing any conclusions on them, but they may perhaps give some hints.

Thus cancer of the stomach maintains its preponderance in men within all groups, though in the case of farmers, female servants, minor officials, and laborers, the women also are comparatively often afflicted. In these classes the women are constantly occupied with the preparing of food, and exposed to the effects of swallowing very hot foodstuffs.

Carcinoma (cutis et) labii shows a strongly marked predilection for farmers (lumbermen included). This points in the same direction as does the frequent occurrence of cancer on the under lip—towards the pipe, and particularly the clay pipe.

Carcinoma uteri finds most of its victims among the laboring, and seafaring and fishing classes, while mammary cancer seems most frequently to occur among the wealthier groups.

These suggestions are, however, offered with every reservation.

7. *Heredity and infection*

There are 2706 of the reports which give information as to the patient's family and domestic relations (parents, children, brothers and sisters, husbands and wives, persons living in the same house, neighbors, etc.). Of these, 1828 (67.5 per cent) deny the occurrence of cancer within this circle, while 878 (32.5 per cent) admit the presence of the disease, with the following distribution:

	Cases
The patient's	
Father was cancerous in.....	250
Mother was cancerous in.....	230
Brother or sister (513 individuals) were cancerous in.....	399
Children (24 individuals) were cancerous in.....	23
Of these	
Father and mother both were cancerous in.....	11
Father, brother, or sister was cancerous in.....	44
Mother, brother, or sister was cancerous in.....	42
Father, mother, brother or sister was cancerous in.....	6
On the other hand, cancer is reported in the patient's	
Husband or wife (<i>cancer à deux</i>) in.....	134
Others living in the same house in.....	174
Others living in the neighborhood in.....	224
Near friends in.....	228

The two groups last mentioned are, however, often rather vaguely defined. Now, all cancer patients have a father and a mother, but not all are married, a fact that of course will give some preponderance to the possibility of finding more cancer patients with cancerous parents than with cancerous mates. But of all the reported cases, only 17.4 per cent were unmarried, and even with addition of these 17.4 per cent double cancer is considerably rarer than cancer in the ancestors and descendants.

It may therefore be concluded that married life with a cancerous person does not seem to convey any greater risk of the development of cancer than does descent from a cancerous father or mother.

Experimental research has shown that a graft of cancerous material will, as a rule, develop in the recipient into a tumor of the same histological type as the original growth. Hence, if cancer in married couples depended upon direct inoculation, it might be expected that the same type of tumor would be found in the two connected cases. Of 125 cases in which this question could be investigated, both mates in 55 couples suffered from cancer of the stomach, in 3 couples from cancer of the lip, in 2 from cancer of the skin, and in 3 from sarcoma. In 63 cases the tumors in husband and wife differed in both type and localization. In no case was cancer of the external genitals found in both.

These figures do not give sufficient support to the supposition of the direct transfer of cancer from one person to another, the less so as the great majority of homogeneous double cases belong to the gastric carcinoma group, in itself so preëminently numerous that the probability of coincidence is great. Besides, married people will as a rule live under identical conditions and partake of the same food.

The reports on cancer affecting parents and offspring show that the localization of the tumors has been the same in all patients in 304 families numbering 667 patients. In the great majority (273 families) the stomach has been the site involved, several times with 3, 4, or 5, and once even with 6 cases in the same family. In 33 cases, cancer of the lip has been the family disease (once with four cases in the nearest family circle), and in 14 families, cancer of the skin.

Sometimes, also, rarer localization with two or more cases in the same family has been reported: carcinoma maxillæ sup. 6 times; c. cutis nasi 2 (in one of the families three members, two sisters and the daughter of one of them); c. canthi ext. 1; c. oesophagi 1 (in four members of the family); c. recti 3; c. vesicæ 1; c. uteri 9; c. ovarii 3; c. prostatæ 1.

In 417 families, on the other hand, embracing 883 cancerous individuals, the localizations were very varying, even twins of the same sex having cancers of different type.

Some of the cases here reported may, perhaps, point in the direction of a family disposition, possibly in the shape of an inherited tendency toward fetal dislocations in the sense of Cohnheim.

8. *Special and local reports*

From a number of practitioners living in the most widely separated parts of the country, the Norwegian Cancer Committee has also received special and detailed reports on the behaviour of cancerous diseases in their various limited regions. Some of these are of great interest but can not here be given. For example, it is not seldom reported that the population in certain circumscribed localities seems to be disproportionately afflicted

with cancer, without it being possible to discover any reason therefore; it has also been reported that the disease after a number of years has disappeared from such places. Again, from the far north, and particularly from Finmarken, several reports agree in stating that the Finlanders (Kvaens) and Lapps are comparatively less often attacked by cancer than the Norwegians themselves, and that immigrants especially from southern parts of the country seem to develop cancer when settling there.

SOME PHASES OF RADIUM ACTION WITH SPECIAL REFERENCE TO THE HEMATOPOIETIC SYSTEM

JOHN A. P. MILLET AND THEODOR MUELLER

From the Hospital of the New York State Institute for the Study of Malignant Disease

Received for publication, August 6, 1917

HISTORICAL

Although a great deal of work has been done in demonstrating the effects on the hematopoietic system of exposure to the various radioactive substances, most of the conclusions reached have been based on the results of experiments on the lower animals, chiefly mice, guinea-pigs, and rabbits. The pioneers in this work were German. Thus, Heineke (2) first demonstrated the profound changes brought about in the spleen and lymph-nodes of mice and guinea-pigs by exposure to the Roentgen rays. No attempt, however, will be made to give a complete review of the voluminous literature: a good summary of all work done prior to 1913 has been provided by Gudzent (1). Suffice it to say that the chief results obtained have been from observations of Roentgen-ray effects, which may be summarised as follows:

1. Destruction of lymphoid cells and deposit of pigment in the spleen (2). The earlier of these effects is explosive in character, and precedes effects on any other group of body cells.
2. Destruction of all forms of bone-marrow cells *in situ* (3).
3. Primary rise of polymorphonuclear count, followed by a drop to a point below normal (4).
4. Steady decline in lymphocyte count (5).
5. Resistance of the red cells to radiation (5).

Although something is known in a vague way among the medical profession about blood-changes in roentgenologists, close

analysis shows that very few other investigations have been made in man, and deductions have sometimes been drawn from an altogether insufficient number of blood-counts. Allen (6), however, was able as early as 1903 to show an immediate slight leucocytosis following x -ray treatment without any appreciable effect on the red cells. Kolde and Martens (7), using mesothorium, demonstrated a primary drop in the red count followed by a return to normal on the fourth day. The most recent work is that of Schweitzer (8), who studied the effect of mesothorium treatment on the blood of patients suffering from cancer of the cervix uteri or vagina. The dosage used was from 2600 to 7700 milligram-hours. His counts were made daily at the same hour and are, therefore, strictly comparable. No counts were made immediately after the application of the mesothorium. His findings may be summarised as follows:

1. Primary leucocytosis followed by a drop to below the original level within twenty-four hours. This subnormal level was maintained until eight weeks after the last treatment.
2. Primary drop in lymphocytes, followed by lymphocytosis.
3. Absence of effect on the red blood cells.
4. Changes in the eosinophile count similar to those in the lymphocyte.

It was this paper which stimulated us to attempt a somewhat more elaborate study of the effects of radium in similar cases, in the hope of throwing some light on the mode of action of the radioactive substances. Schweitzer attributes these blood changes to the action of a supposed "Roentgen-toxin" or to the production of toxic substances of unknown origin, and regards the leukopenia as an unfavorable effect.

INTRODUCTORY

The object of the following study was to determine the immediate and remote effects of radium on the activity of the blood-forming organs, as demonstrated by the numbers of the various formed elements found in the circulating blood at definite stated intervals after the radium application, and with special reference to changes in the leucocytes.

For this purpose, ten patients were chosen who had squamous-cell carcinoma of the cervix uteri and vagina, some hitherto untreated, others with recurrences after a previous operation. The number of applications varied in the different cases, as did also the intervals between applications. Some of the patients received x -ray treatment in addition to radium. In view of this fact, the remote effects of treatment may be due to the cumulative effect of both x -ray and radium. The immediate changes, however, following radium application may be ascribed to this agent alone, especially since these changes were present in most marked degree in those patients who had had no previous x -ray treatments.

TECHNIQUE

The radium was applied directly to the tumor mass in a brass cylindrical applicator,¹ which, for purposes of cleanliness, was wrapped in a rubber finger cot. No further screening was used. The applicator was introduced through a speculum and packed in place with cotton. In applications to the vaginal wall the healthy sides were protected by placing the applicator in a half-cylinder of lead 2 mm. in thickness, adhesive plaster and rubber being used to absorb the secondary rays.

The dose employed was 50 mgm. radium element in the form of radium bromide. The time used was thirty hours for each application whose immediate effects were studied, with two exceptions (case I, August 4, 1916, eighteen hours, and case VIII, November 20, 1916, twenty-six hours). Applications whose immediate effects were not studied varied from three to forty-eight hours in duration. The interval between treatments varied from twelve to sixty-two days, the usual interval being two to three weeks.

The patients were kept in bed during the whole course of the treatment. On the first day of the application the blood was counted in the early morning, between 5 and 9 a.m., usually at 5.45 a.m. The radium was applied immediately afterwards and

¹ The brass cylinder was the usual type distributed by the Radium Chemical Company of Pittsburgh, Pa., and styled "Universal Applicator, Type B."

counts were then made at the following intervals: $\frac{1}{2}$ hour, 1 hour, 2 hours, 4 hours, 6 hours, 9 hours, 12 hours, and then daily. The patient was given no breakfast, and took a light luncheon after the 6-hour count, and supper after the 12-hour count, so that there was always two and one-half hours between any meal and the next following blood-examination. The daily counts were made between 10 a.m. and 11 a.m., three or four hours after breakfast. The following counts were made on each occasion: Total white count and differential count (stained smear or unstained specimen used for white count²); in five cases a red cell count was also made (nos. VI, VII, VIII, IX, and X) and in three a platelet count (nos. VI, IX, and X).

Observations on any given series were made as far as possible by a single individual in order to eliminate individual differences in technique.

TREATED PATIENTS

The following changes were found to take place in the period immediately following the radium application.

A. Total white count

1. A drop in the total white count. This usually was found to occur within the first half-hour, but sometimes did not reach its lowest point until six hours after the radium application. In one instance no drop was recorded, but here the half-hour count was not taken (case IX). Counts made on this patient during a subsequent treatment showed a primary drop during the first half-hour. In two cases (VIII and X) the drop was preceded by a slight temporary rise. In one case (V) the primary drop was succeeded after a temporary rise by a secondary drop, which reached its lowest point six and fifteen hours after the application during two successive treatments.

² It was found by careful comparison of several simultaneous counts that there was usually no difference worth consideration between results obtained from stained and those from unstained specimens. Occasionally the difference was fairly distinct, but this did not occur more frequently than it does in counting two separate hundreds in one stained specimen.

2. A return of total white count to approximately its former level. This occurred on nine occasions within the first six hours, on seven occasions between six and twelve hours, and on two occasions between twelve and nineteen hours after the application.

3. A compensatory rise of the total white count, in some instances to a point considerably higher than before the radium application. This phenomenon occurred as follows:

Twice, two to three days after the application.

Three times, twenty-four to twenty-six hours after the application.

Four times, twelve to twenty-four hours after the application.

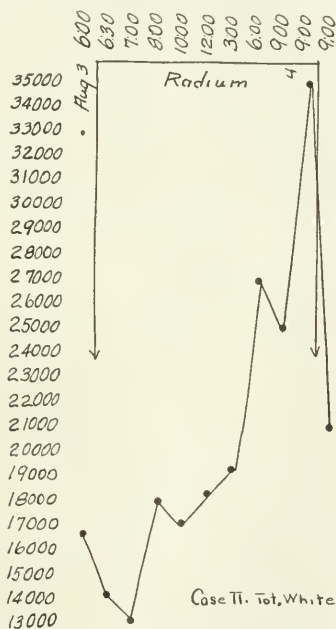
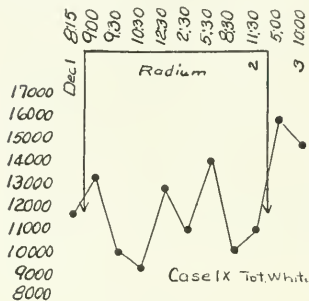


CHART I. EARLY CHANGES IN TOTAL WHITE COUNT

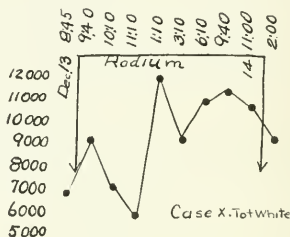
Twice, within twelve hours after the application.

Seven times, no such rise occurred.

Another striking point that comes out on study of the charts is that in cases where the early response to radium application is not altogether typical, for example, when the primary drop in total



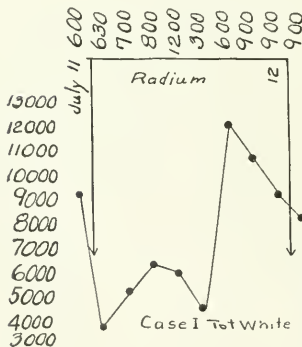
First treatment



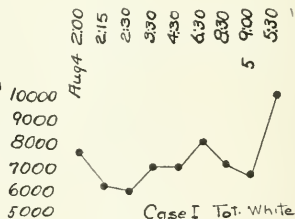
Second treatment

CHARTS 2A AND 2B. ATYPICAL REACTION REPEATED DURING SUBSEQUENT TREATMENT

Note preliminary rise before usual fall



First treatment



Second treatment

CHARTS 3A AND 3B. MODIFIED REACTION OF TOTAL WHITE COUNT DURING SUBSEQUENT TREATMENT

white count is delayed, or unusually well sustained, or preceded by a temporary rise, the same behavior is noted during a later application (see cases V, VIII, and X).

Finally, it is also worthy of note that in one case (case I) the response of the polymorphonuclears to the radium application was not so marked following the later application as it was following the first. It would seem in this case that the power of the hematopoietic system to react to what would usually be an efficient stimulus had been diminished, either by frequent radium applications in the past or by the advance of the disease itself.

Observations on the stained smear, which will be considered later, seem to show that this failure to react is due to lasting damage to the hematopoietic system from the primary radium application.

B. Total polymorphonuclear count

This ran throughout so closely parallel to the total white count that all that has been said above in regard to changes in the total count applies also to changes in the polymorphonuclear count.

C. Total lymphocyte count

1. No characteristic change was found to occur in the total lymphocyte count immediately after the application of radium. There was more frequently a drop than a rise, but the degree of change in either case was usually so slight and the duration of the change so variable that it need not be considered.

2. No characteristic change in the total lymphocyte count could be demonstrated to occur during the first two weeks following treatment. The largest drop was from 7800 to 1000 (case IV) at the end of nine days, the largest rise being from 1200 to 4200 at the end of thirteen days (case VI).

3. In most cases, the total lymphocyte count varies to some extent in harmony with the variations of the total white count. This behavior is most noticeable, as a rule, when the variations in the total white count are considerable (cases III and IV), although it is sometimes noticed when variations are rather small

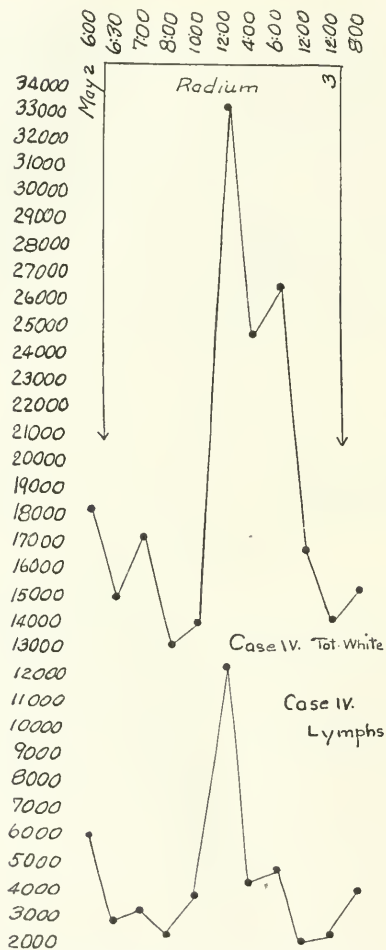


CHART 4. SHOWING EARLY CHANGES IN TOTAL LYMPHOCYTE COUNT COMPARED WITH CHANGES IN TOTAL WHITE COUNT

(case V). There are exceptions, however, to this general statement, as for instance in case VI, where the variations in the total white count are distinct, while the total lymphocyte count shows only a slight variation from one time to another.

D. Total large mononuclear count

No constant variations were found in the total mononuclear count.

E. Percentage values of the various cells

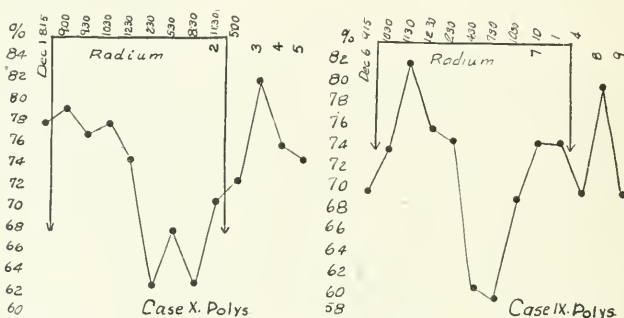
These were studied in stained smears made during and after nine radium treatments in five patients. In all these cases the blood showed some pathological findings before treatment was begun, as, for instance, absence of eosinophiles, immature forms of white and red cells, and changes in the form of the red cells. In two patients the percentage of polymorphonuclears and lymphocytes was within normal limits: in the other three there was pronounced neutrophilia and lymphopenia.

1. *Neutrophiles.* In eight out of the nine treatments there is first a drop in the neutrophilic curve, preceded in five instances by a brief preliminary rise lasting from one-half hour to nine hours after the radium application. This drop lasts from one to twelve hours after the beginning of treatment, and is immediately followed by a rise to a point which is always above the level noted before the treatment was begun, in some instances as high as 93 per cent. This rise usually attains its highest point within forty-eight hours after the time of application, though in one instance this was not reached until the third day. During the days that follow there is a gradual return of the curve to its normal level, which is usually reached by the fifth to the eighth day. In one patient (case X) the drop continued until the second treatment was begun (eleven days) and then continued still further until well below the normal level.

In one treatment no definite change in the neutrophile percentage could be demonstrated. This case showed a very pronounced neutrophilia and lymphopenia.

To summarize, the following points may be made:

- (1) There is a drop in the neutrophile percentage, sometimes preceded by a temporary rise. This drop is not below the lower limit of normal.
- (2) After twelve hours, there is a compensatory rise to a point above the upper limit of normal.
- (3) The curve returns to the normal level in five to eight days.

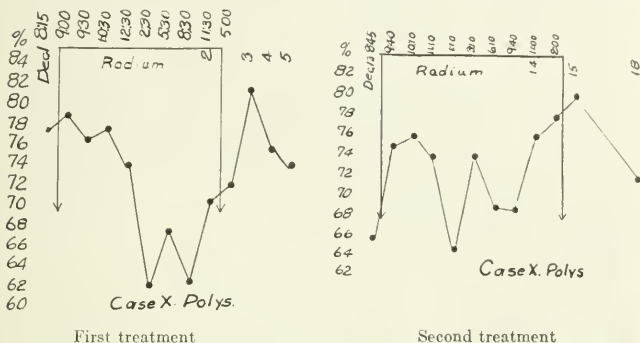


CHARTS 5A AND 5B. EARLY CHANGES IN POLYMORPHONUCLEAR PERCENTAGES.

Of interest in this connection is the behavior of the myelocytes and of those neutrophils which are in the transitional stage between the myelocyte and the polymorphonuclear form—the so-called “metamyelocytes” of Pappenheim (9). These latter cells could be demonstrated in the blood of all the patients before treatment was begun, indicating, probably, some irritation of the bone-marrow due to the toxins resulting from the disease. During radium treatment, however, they showed a decided increase simultaneous with the rise in neutrophile percentage. In cases VII and X the rise in metamyelocytes preceded and heralded the rise of neutrophils. The myelocytes, likewise, were observed to increase in numbers. In four out of the five patients these were demonstrated in the blood in small numbers before treatment, but during treatment they were found much

more frequently. This was especially the case in case X, who, during her second treatment, showed 2 per cent myelocytes in the blood. It would seem, then, that the radium has a definite irritant action on the bone-marrow.

The behavior of the neutrophile count during subsequent treatments is somewhat modified. Four out of the five patients were given several treatments. Of these case IX showed simply an irregular drop in neutrophiles during the second treatment, Case VI, who was treated five times, and whose blood was studied



CHARTS 6A AND 6B. CHANGED REACTION OF POLYMORPHONUCLEARS DURING SUBSEQUENT TREATMENT

during the first and fourth treatments, showed no appreciable change in the neutrophile curve during the fourth treatment. It is fair to state in this connection that at this time she showed a marked neutrophilia (93 per cent) and lymphopenia (5 per cent). In case X the effect on the curve was not quite so marked during the second as during the first treatment.

In general, then, it may be said that the radium effect is more marked during the first application than during later treatments.

2. *Lymphocytes.* These likewise showed a constant percentage curve in eight out of the nine treatments studied. In case X no such response could be demonstrated, although even here

the curve has one thing in common with those of the other cases, namely the drop in relative lymphocyte count at the end of the 30-hour treatment. The changes noted in the other eight instances are as follows:

(1) An increase in the count, reaching its maximum in from two to twelve hours, and preceded in five instances by a brief preliminary drop. In the two cases which showed a normal percentage before treatment the count rose to 30 per cent, while in those which had showed marked lymphopenia the highest point never exceeded the upper limit of normal, and in some instances did not reach the normal level at all.

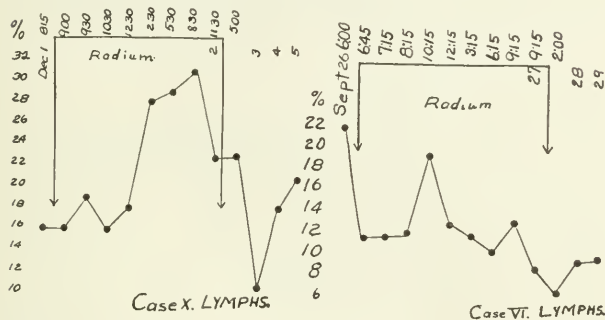
(2) After from four to twelve hours there begins an irregular drop, which in seven instances reaches its lowest point before the end of the treatment (thirty hours). In one instance, the lowest point is not reached until the next day, while in the ninth instance (case IX) the drop continues for eight days. In every instance the low point is well below the lower limit of normal. In those patients who showed a normal blood picture before treatment the percentage fell as low as 9 per cent, while in those with previous lymphopenia it even reached 4 per cent. In one case the low point was still slightly above the level noted before treatment (case VI, second treatment), but the blood in this case was highly abnormal, showing an extreme lymphopenia (5 per cent) before treatment.

It seems reasonable to infer that this drop in the percentage of lymphocytes is an expression of actual damage done to the lymphatic system, a supposition which is borne out by the finding of numerous pyknotic and vacuolated lymphocytes in the blood-smears taken during this period. These degeneration forms first appear six to ten hours after the beginning of treatment and remain in the smear until its end (thirty hours), an observation which agrees with those of Heineke (2).

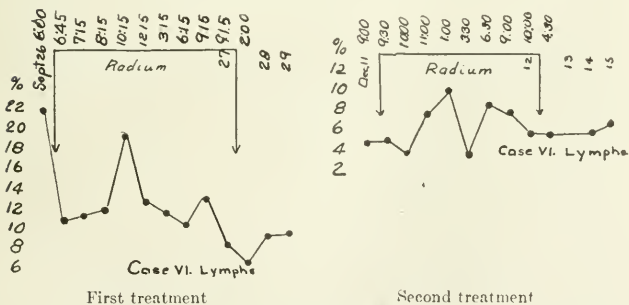
(3) Immediately or shortly after this period of depression there is a secondary rise in the lymphocyte curve, even in those cases which had showed a marked lymphopenia. In six instances this rise continues to a point slightly above the level noted before treatment, once to a point just below it. In one case only (case VI) it is impossible to demonstrate this rise.

To sum up, the lymphocyte curve is approximately the reverse of the neutrophile curve, and shows:

- (1) A rise, sometimes to a point as high as 30 per cent, and preceded occasionally by a preliminary drop.
- (2) A sharp drop after four to ten hours to a point well below the normal level.
- (3) A return to the original level after several days.



CHARTS 7A AND 7B. EARLY CHANGES IN LYMPHOCYTE PERCENTAGES IMMEDIATELY FOLLOWING APPLICATION

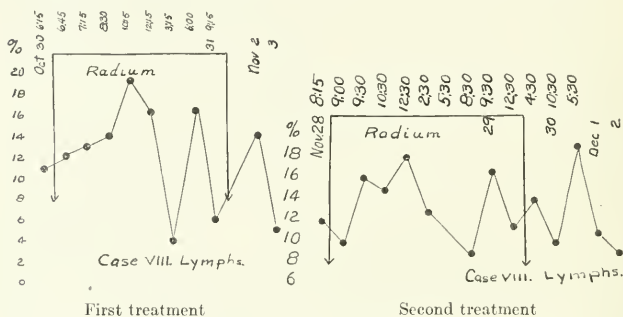


CHARTS 8A AND 8B. CHANGED REACTION OF LYMPHOCYTES DURING SUBSEQUENT TREATMENT

As in the case of the neutrophils, the lymphocytes show a modified reaction to repeated treatments. More than one treatment was given in cases VI, VIII, IX, and X.

In case VI, five treatments were given and the blood was studied during the first and fourth. The influence of the fourth treatment was much less marked than that of the first.

In case VIII the curve showed about the same course during both treatments, although the difference between highest and lowest points is less during the second than during the first.



CHARTS 9A AND 9B. MODIFIED REACTION OF LYMPHOCYTES DURING SUBSEQUENT TREATMENT

In case IX, however, the influence of the second treatment was more protracted and intense than that of the first. The difference between high and low points was greater during the second treatment, the figures being 32 vs. 8 as opposed to 27 vs. 9 during the first treatment. After the first treatment the original level was again reached after forty-eight hours and the following weeks showed a rise in average lymphocyte count, while after the second the normal level was not reached until the twelfth day, and the average count of the following weeks (21 per cent) was less than the average count of the weeks following the first treatment (24 per cent). In this case, however, constitutional effects must be taken into consideration, because twenty-one

days after the second treatment there appears a terminal sharp drop in relative lymphocyte count.

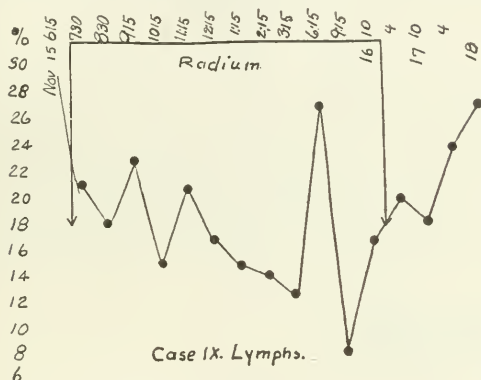


CHART 10A. INTENSIFIED REACTION OF LYMPHOCYTES DURING SUBSEQUENT TREATMENT
First treatment

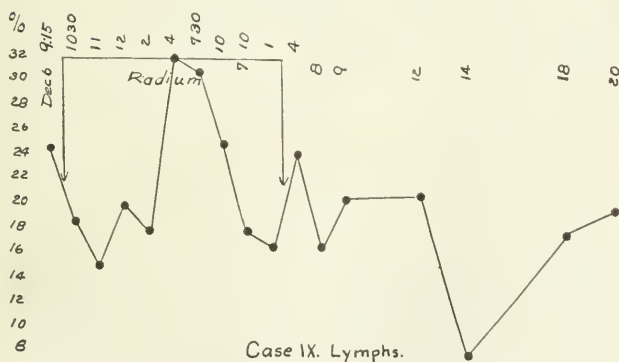
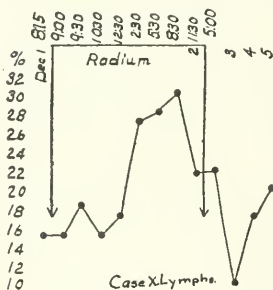


CHART 10B. INTENSIFIED REACTION OF LYMPHOCYTES DURING SUBSEQUENT TREATMENT
Second treatment

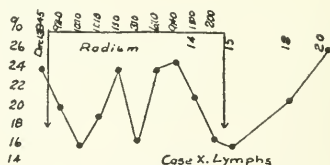
In case X the reaction of the lymphocytes to the treatment is decidedly stronger during the first application than during the second, as evidenced by these figures:

		per cent
First treatment	Highest.....	31
	Lowest.....	11
Second treatment	Highest.....	24
	Lowest.....	16

As in case IX, however, the effect is more protracted after the second treatment than after the first, the original level being again reached on the second day after the first treatment and not until the seventh day after the second. In the period following these treatments the count rose to 24 per cent after the first, and 26.7 per cent after the second.



First treatment



Second treatment

CHARTS 11A AND 11B. MODIFIED REACTION OF LYMPHOCYTES DURING SUBSEQUENT TREATMENT

In general, then, it may be said that the lymphocytic reaction, numerically considered, is less marked with subsequent treatments than with the original one, although the "depressor" effect may be more lasting after the later treatments. This fact seems to indicate a permanent damage to the lymphopoietic system from the first treatment, rendering it incapable of its normal reaction to a given stimulus and slower to recover from the effects of that stimulus. In cases VI and X there were

greatly increased numbers of pyknotic and vacuolated lymphocytes during the later treatments, which may be taken as confirmatory evidence of the increased damage to the lymphopoietic system.

3. *Large mononuclears.* During seven treatments there was noted a preliminary rise of the large mononuclear percentage, the highest point being reached four to twenty-six hours after the application. In three instances during a first treatment and

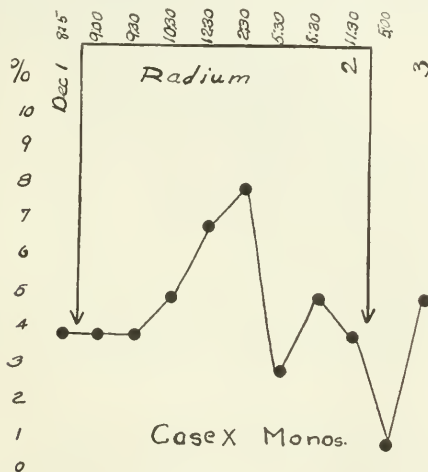


CHART 12. EARLY CHANGES IN MONONUCLEAR PERCENTAGES

once during a later treatment the figure rose above the upper limit of normal, but in all other instances the percentage remained within normal limits. This rise was followed by a drop which persisted until the end of the treatment on the day following. In two instances it continued to the third and fourth day respectively. Twice this secondary drop occurred without any preliminary rise. In all cases the curve fell to a point well below normal, once even getting down to 1 per cent. This drop

in turn was followed by a rise to the original level or above, lasting from one to nine days after treatment.

Repeated treatments showed absence or diminution in intensity of this effect. The curve of the large mononuclears follows so closely that of the lymphocytes as to favor the theory of their lymphatic origin.

4. *Eosinophiles*. Prior to treatment all patients showed an absence or decrease in number of the eosinophiles. During treatment there was noted in three cases a rise at varying periods, in one instance as high as 6 per cent. In the other cases no defi-

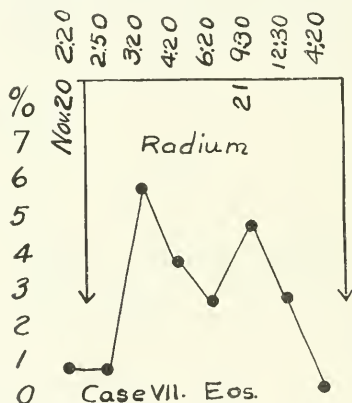


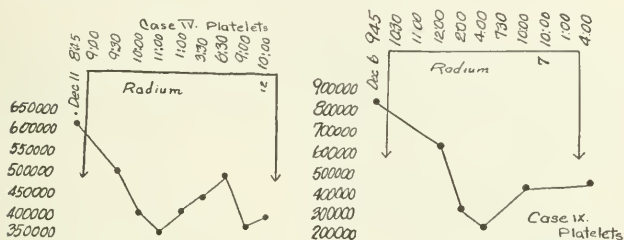
CHART 13. EARLY CHANGES IN EOSINOPHILE PERCENTAGE

nite influence was demonstrable. These findings are directly contrary to those of Schweitzer (8), who noted a disappearance of eosinophiles during and after treatment, with reappearance two to three weeks later.

F. Platelet count

The platelets were counted during four treatments on three patients (VI, IX, and X). In three instances there was an immediate drop; in two of these the drop was distinct and

lasted two and six hours respectively; in the third case the drop was very slight, lasting only an hour, and was succeeded by a slight steady rise for the succeeding two days. In one instance there was practically no change, although there was a slight steady drop for the first nine hours followed by a return to normal.



CHARTS 14A AND 14B. EARLY CHANGES IN PLATELET COUNT

In general, then, it may be said that the platelets, like the polymorphonuclear cells, show a tendency to an immediate though often a slight decrease in absolute numbers.

G. Hemoglobin

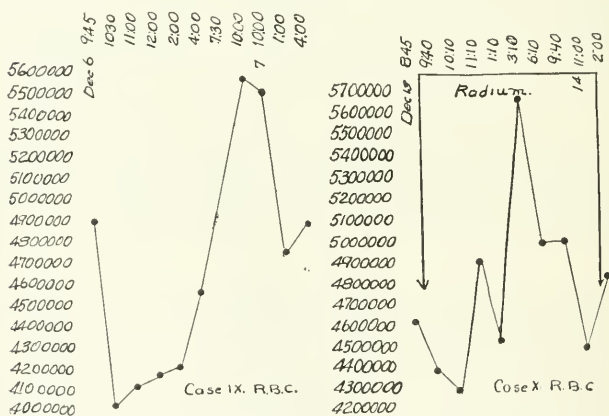
This was estimated on four occasions (cases VI, VIII, IX, X) during treatment, but showed no change of any importance.

H. Red cell count

The red cells were counted during seven treatments in five cases; in every instance there was considerable fluctuation in the figures. All cases, however, showed a definite increase in the count, at some period within the first three days following the time of application, to a level well above that of the count taken before the radium was applied. Thus:

CASE	BEFORE TREATMENT	LENGTH OF TIME	HIGHEST COUNT
VI	3,250,000	3 days	4,000,000
VII	4,300,000	22 hours	5,350,000
VIII	5,000,000	33½ hours	6,100,000
VIII	4,900,000	12 hours	5,600,000
IX	4,150,000	12 hours	5,000,000
IX	4,600,000	6 hours	5,800,000
X	4,500,000	½ hour	5,100,000

In five out of the seven instances there was a preliminary drop in the count, lasting from one-half hour to four and one-half hours in the different cases. This was especially marked in case X, which did not show so evident a secondary rise as the other cases.



CHARTS 15A AND 15B. CHARTS SHOWING CHANGES IN RED BLOOD COUNT
SPECIMEN CHARTS

I. Blood-pressure

Blood-pressure estimations were carried out simultaneously with the blood-counts in five instances (cases VI, VII, VIII, IX, and X) with the idea of trying to determine whether any

evidence could be found for the formation of any substance having a lowering effect on the blood-pressure. Cholin has been shown to be one of the products of radium action and has been supposed by some authors to be responsible for the beneficial effect of radium on tumors (10). This substance exerts a depressor effect when introduced into the animal organism. The following table will show the results noted:

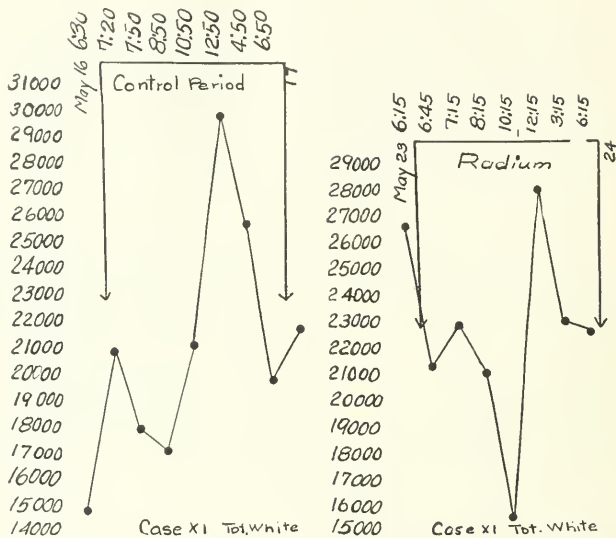
Blood-pressure readings—systolic and diastolic

TIME OF READING	CASE VI	CASE VII	CASE IX	CASE XB	CASE XA	CASE VIII
Before treatment.....	148-82	148-84	152-88	112- 80	122-86	114-68
$\frac{1}{2}$ hour after application.....	136-68	150-86	142-82	110- 82	120-88	112-68
1 hour after application.....	130-66	138-80	126-78	114- 86	126-90	110-66
2 hours after application.....	142-80	142-78	136-80	110- 80	114-86	116-66
4 hours after application.....	130-66	140-76	144-78	116- 86	122-90	116-68
6 hours after application.....	134-66		136-76	112- 80	120-82	114-66
9 hours after application.....	128-66		140-84	120-100	126-86	120-70
12 hours after application.....	128-66		138-84	116- 90	112-80	122-78
18 to 25 hours after application.....	122-64	130-78	136-82			120-68
26 to 36 hours after application.....	132-68	138-80	132-78		126-86	106-66

From this table it will be seen that in cases VI and IX, and to a less extent in case VII, there is a tendency towards a fall in blood-pressure. The others, however, show no such tendency. It seems fair to assume that the small drop noted in the three first cases may be explained as the result of complete rest in bed, especially as the three patients in question were ambulatory at the time of treatment while cases VIII and X were bed-ridden.

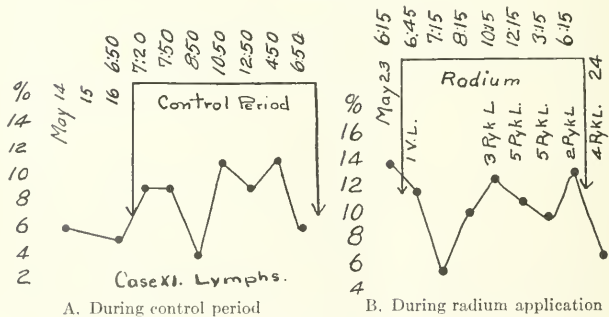
CONTROL CASE

In order to control these observations, we selected an eleventh patient who also had an inoperable carcinoma of the cervix uteri, and counted her blood under exactly the same conditions as prevailed with the treated cases. While there was considerable fluctuation in the counts during the day, the features noted above were not reproduced. Thus, for instance, we find no initial drop in the total white count during the con-

A. Before any treatment
(Control period)

B. During radium application

CHARTS 16A AND 16B. CHANGES IN TOTAL WHITE COUNT



A. During control period

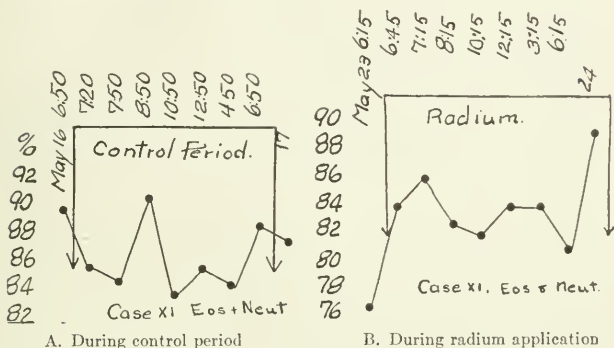
B. During radium application

CHARTS 17A AND 17B. CHANGES IN LYMPHOCYTE PERCENTAGE

Note: V.L. = Vacuolated lymphocyte; Pyk.L. = Pyknotic lymphocytes

trol period, while during the radium treatment the usual curve is noted.

In a similar way, the percentage counts of lymphoid cells



CHARTS 18A AND 18B. CHANGES IN POLYMORPHONUCLEAR PERCENTAGE

Case XI Myel. + Meta myel.

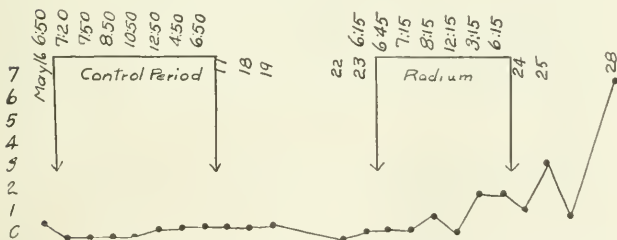


CHART 19. COMBINED MYELOCYTES AND METAMYELOCYTES DURING CONTROL PERIOD AND DURING RADIUM TREATMENT

and polymorphonuclears show no characteristic reaction during the untreated period, while during the radium treatment the typical curves are reproduced.

Most striking of all is the number of myelocytes and metamyelocytes found during radium treatment, as contrasted with their absence during the control period (see chart 19).

It is further noteworthy that pyknotic lymphocytes were abundant during the radium treatment, indicating destruction of the lymphoid elements, and completely absent during the control period (see chart 17B).

From a study of this case, therefore, it would seem fair to assume that the changes noted are indisputably dependent on the action of the radium, and not mere variations within physiological limits.

The remote effects of radium treatment as shown by the counts made on these nine patients may now be analysed. For the purpose of avoiding false deductions, the cases will be divided into those which were treated with radium alone (group 1) and those which had x-ray treatments in addition (group 2). In the former class there were three patients in the group studied: two of these were followed for only twenty-four and forty-six days respectively, but the third was followed up till the time of death.

In the latter class there were six cases: of these one was followed for thirty-four days, one for fifty-four days, one for fifty-seven days, and the remaining three until the time of death.

All intervals will be reckoned from the date of the first radium treatment, irrespective of whether that particular treatment was used for the purpose of studying the immediate blood changes.

In the following tables the blood changes are summarised by charting the various counts at arbitrary intervals. Unless otherwise stated the figures given are the average figures calculated from two to five counts on successive or almost successive days. This procedure was adopted to avoid the possibility of recording some single atypical count as representing the average count of the period. In some instances, only one count was made at a given period, so that sometimes it has been impossible to take the average of several counts. These instances are noted.

Tables Showing Remoter Blood-Changes Following Radium Treatment³

Group 1

CASE III	TOTAL WHITE BLOOD CELLS	TOTAL POLY- MORPHO- NUCLEARS	TOTAL LYMPHO- CYTES	PER CENT POLY- MORPHO- NUCLEARS	PER CENT LYMPHO- CYTES
Before treatment.....					
3 weeks after.....	11,000	7,600	2,100	69	19
4 weeks after.....	10,000 ¹	8,400 ¹	1,200 ¹	84 ¹	12 ¹
5 weeks after.....	12,000	9,850	1,500	82	12
6 weeks after.....	8,500	4,500	2,400	53	28

In this group of cases the blood-changes may be summarised as follows:

Case III. (1) A slight decrease in the total white count, most marked at the end of six weeks.

(2) A sharp change in the relative polymorphonuclear and lymphocyte count from four to five weeks after treatment, due chiefly to a marked decrease in the absolute lymphocyte count.

(3) A reversal of this process at the end of six weeks, with marked decrease of the absolute and relative polymorphonuclear count and increase of the absolute and relative lymphocyte counts to a point slightly above normal. Comparison of these figures with those of three weeks previous shows the change to be primarily due to a depression of the polymorphonuclear count rather than to a lymphocytic increase.

To summarise the findings briefly, it appears that there was in this case first a depression of the lymphocytic series lasting from four to five weeks after treatment, and later a depression of the polymorphonuclears, appearing first after six weeks and accompanied by a recovery of the lymphocytic series.

Case IV. (1) A moderate decrease in the total white count, the lowest point being reached in four weeks, after which time the count begins to rise again.

³ Blood-counts marked with the figure 1 throughout these tables are single counts. All others represent an average drawn from two or more counts on successive or almost successive days.

CASE IV	TOTAL WHITE BLOOD CELLS	TOTAL POLY- MORPHO- NUCLEARS	TOTAL LYMPHO- CYTES	PER CENT POLY- MORPHO- NUCLEARS	PER CENT LYMPHO- CYTES
Before treatment.....	19,500	12,000	4,700	67	24
2 weeks after.....	13,500	7,800	3,600	58	27
3 weeks after.....	13,000	8,200	3,650	63	28
4 weeks after.....	10,000	5,300	2,800	53	28
5 weeks after.....	13,000	9,000	2,750	69	21
6 weeks after (approximately).....	15,000	11,000	2,700	73	18

(2) A steady decrease in the total lymphocyte count, the percentage count increasing slightly for four weeks and then beginning to drop. In this case the fall in total lymphocytes during the first four weeks is not nearly so marked as the fall in the polymorphonuclear count and is roughly proportional to the fall in the total white count. Furthermore, during the fourth to sixth week the decrease in the total and relative lymphocyte counts is subordinated in importance to the rise in the polymorphonuclear count.

To summarise, this chart shows a primary depression of the polymorphonuclear count lasting four weeks, followed by a compensatory stimulation. Accompanying this effect there is a slight but progressive depression of the lymphocytic series, reaching the lowest point at the end of six weeks. These findings are a contrast to those noted in case III.

CASE VI	TOTAL WHITE BLOOD CELLS	TOTAL POLY- MORPHO- NUCLEARS	TOTAL LYMPHO- CYTES	PER CENT POLY- MORPHO- NUCLEARS	PER CENT LYMPHO- CYTES
Before treatment.....	29,000	23,200	4,300	80	15
2 weeks after.....	33,500	28,100	3,350	84	10
1 month after.....	21,000	17,500	2,500	83	12
1½ months after.....	18,000	15,300	1,980	85	11
2 months after.....	28,000	23,250	3,400	83	12
2½ months after.....	20,500	17,800	2,050	87	10
3 months after.....	31,000	28,850	1,250	93	4
3½ months after.....	50,000	49,000	500	98	1
4 months after.....	130,000	127,400	1,625	98	1

Case VI. (1) A fall in the total white count and the total polymorphonuclears during the first one and one-half months

after a brief preliminary rise, followed by an irregular slight rise until three months and a sharp terminal rise from three and one-half to four months.

(2) A steady drop in the total lymphocyte count and a more gradual drop in the percentage count. The steady fall is interrupted at two months by a temporary rise in the total count, but at this period the rise in the total white count is proportionally almost as high.

(3) While at two and one-half months there is a temporary decrease in the total polymorphonuclear count, the rise in the polymorphonuclear percentage begins at that time and presages the later rise in the total polymorphonuclear count.

To summarise, then, we have in this case a drop in all white blood cells in about the same proportion during the first one and one-half months. The lymphocytic drop, however, starts at two weeks, the polymorphonuclear drop at one month. After the first one and one-half months there is a further decrease in the lymphocytes and a disproportionately large increase in the total white count and polymorphonuclears, ending in an enormous leucocytosis during the two weeks preceding death.

DISCUSSION OF RESULTS

Group 1

The remoter changes in blood-findings may be divided into the early and late changes.

Early changes. (1) All three charts show early depression of both lymphocytic and polymorphonuclear counts. In two of the three cases the lymphocytes show the drop earlier than the polymorphonuclears; in the third case the drop in both is simultaneous. The time when these phenomena appear is not constant, the lymphocytes showing a drop at two weeks in two cases and at four weeks in the third case. In the third case, however, it is not possible to say that the drop did not appear earlier, inasmuch as no count was taken until three weeks after the first treatment.

In the same way, the polymorphonuclears show the drop at two, four, and six weeks repeatedly in the three charts.

(2) One case (III) shows a recovery of the lymphocytes at the sixth week, another (VI) at two months. The third case (IV) shows no such reaction up till the last count (at six weeks). It is interesting to note in this connection that case III lived nine months after treatment was begun.

In case VI the lymphocytic recovery is accompanied by a sharp reaction in the polymorphonuclear series, for, although the absolute lymphocyte count rises to a point above normal, the total white count also rises and the relative lymphocyte count remains about as before. This patient died four months after treatment was begun. Case IV shows at six weeks a beginning leucocytosis and a further depression of the lymphocytes, changes which point to impending death. The patient died four months after treatment was begun.

Late changes. (1) The most noteworthy late change is the terminal leucocytosis. In case VI this is found to be very marked and is characterised by enormous increase in the absolute polymorphonuclear count together with moderate decrease in the absolute lymphocyte count, factors which combine to give a very low relative lymphocyte count and correspondingly high relative polymorphonuclear count.

(2) Before the leucocytosis sets in another change may be noted. This is the change in relative counts from two and one-half to three months after the institution of treatment, the lymphocytes decreasing, the polymorphonuclears increasing. This apparently is the signal for the collapse of the patient's resistance and presages the terminal stage.

It may now be asked with reason: How big a part does the radium play in causing these changes, and how far are they due to the disease process? In answer it would seem fair to claim that the radium is responsible for the early changes, that is, the fall in lymphocytes and polymorphonuclears; but that the later changes, i.e., the marked change in relative counts and the terminal leucocytosis, represent the breakdown of the body's resistance and its final effort to combat the flood of

toxic products circulating in the blood stream. In this sense, the lymphocyte may be said perhaps to act as a rough indicator of the bodily resistance toward the later stages of the disease before the terminal leucocytosis has set in. Its practical value in this connection, however, is small, because it does not show any characteristic change until the break-down of the patient is clinically all too obvious.

Tables Showing Remoter Blood-Changes Following Combined x-ray and Radium Treatments

Group 2

CASE I	TOTAL WHITE BLOOD CELLS	TOTAL POLY- MORPHO- NUCLEARS	TOTAL LYMPHO- CYTES	PER CENT POLY- MORPHO- NUCLEARS	PER CENT LYMPHO- CYTES
Before treatment.....					
2½ months after.....	9,500 ¹	5,900 ¹	2,450 ¹	62 ¹	26 ¹
5 months after.....	7,500	4,850	1,350	65	18
6 months after.....	7,400	3,750	2,200	51	30
Several short treatments: Heavy treatment begun just before next count					
11 months after.....	6,200 ¹	4,850 ¹	875 ¹	78 ¹	14 ¹
12 months after.....	13,500	11,350	1,200	84	9
12½ months after.....	14,500	12,200	1,450	84	10

Case I. (1) The total white count shows a steady drop, lasting until the eleventh month, followed by a marked rise from the twelfth to thirteenth months.

(2) The lymphocytes show a sharp drop between two and one-half and five months, followed by a compensatory rise at the sixth month, while the polymorphonuclears show only an absolute decrease after the first five months, the relative decrease appearing first at six months, at which period it is overshadowed by the lymphocytic increase.

(3) The lymphocytes show a marked drop at the eleventh month which accounts for the greater part of the drop in total white count. The percentage of polymorphonuclears correspondingly makes a great jump, although the absolute number shows only a slight increase.

(4) At the twelfth month there is a sharp increase in total white count and polymorphonuclear count, which explains the increase in polymorphonuclear percentage and the decrease in lymphocyte percentage.

To summarise, this chart shows at five months a primary decrease in lymphocytes, followed by a temporary compensatory rise (at six months) and, finally by a gradual relative decline. The corresponding fluctuation in the total white count is determined largely by the lymphocytic changes until the twelfth month, when there appears a polymorphonuclear leucocytosis. This final change is presaged as in case VI at eleven months by a change in relative counts without any increase in total white count.

CASE II	TOTAL WHITE BLOOD CELLS	TOTAL POLY- MORPHO- NUCLEARS	TOTAL LYMPHO- CYTES	PER CENT POLY- MORPHO- NUCLEARS	PER CENT LYMPHO- CYTES
Before treatment.....					
5 months after.....	16,500 ¹	13,350 ¹	1,650 ¹	81 ¹	10 ¹
Heavy treatment instituted one week later					
6 months after.....	19,000	15,600	2,300	82	12
7 months after.....	18,000	15,500	1,800	86	10
8 months after.....	21,000	18,500	1,700	88	8
9 months after (approximate time of death).....	44,000	41,350	1,750	94	4

Case II. This chart shows: (1) A steady increase in total white count.

(2) A gradual increase in percentage of polymorphonuclears.

(3) A gradual decrease in percentage of lymphocytes except for one period (six months) when there is a slight temporary rise, accompanied by a rise in actual numbers. This represents a transitory stimulation following the first trial of a thirty-hour treatment. In this chart, again, the terminal leucocytosis is foreshadowed by the changing percentage counts. This change is due to a steady increase in the absolute polymorphonuclear count, the absolute lymphocyte count remaining practically stationary throughout.

CASE V	TOTAL WHITE BLOOD CELLS	TOTAL POLY- MORPHO- NUCLEARS	TOTAL LYMPHO- CYTES	PERCENT POLY- MORPHO- NUCLEARS	PER CENT LYMPHO- CYTES
Before treatment.....	12,000	8,750	2,150	73	18
4 weeks after.....	9,000	7,000	1,250	78	14
15 weeks after.....	5,500 ¹	4,100 ¹	800 ¹	75 ¹	14 ¹
19 weeks after.....	12,500 ¹	10,000 ¹	2,000 ¹	80 ¹	16 ¹
22 weeks after.....	15,500	14,400	950	93	6
27 weeks after.....	Died				

Case V. The chart of this case shows: (1) A fall in total white count continuing until fifteen weeks after treatment was instituted, after which time the terminal rise begins.

(2) The lymphocytes show a primary drop after four weeks which is proportionally greater than the rise of polymorphonuclears. At fifteen weeks the polymorphonuclears show a more distinct drop than the lymphocytes.

(3) At nineteen weeks both groups of white cells show an approximately proportional rise, while at twenty-two weeks the polymorphonuclears show a rise, though this is not so marked as the drop in lymphocytes. This is a beginning terminal leucocytosis. If later counts had been made the polymorphonuclears would probably have shown a large relative increase.

Summary. This chart shows a primary lymphocytic drop followed by a drop in polymorphonuclears, the former starting at four weeks, the latter at eighteen weeks. The relative counts show an almost steady increase of polymorphonuclear cells and an almost steady decrease of lymphocytes. The terminal leucocytosis is preceded as usual by a definite though gradual change in the relative proportion of polymorphonuclears and lymphocytes.

Case VIII. This chart shows: (1) Irregular fluctuation of total white count and of total polymorphonuclear count.

(2) Depression of lymphocytic series from the second to the third week, as evidenced by a primary drop in total lymphocyte count despite an increase in total white count, and by the decrease in relative count at the third week in spite of the drop in total white count at that time.

CASE VIII*	TOTAL WHITE BLOOD CELLS	TOTAL POLY- MORPHO- NUCLEARS	TOTAL LYMPHO- CYTES	PER CENT POLY- MORPHO- NUCLEARS	PER CENT LYMPHO- CYTES
Before treatment.....	8,600 ¹	6,500 ¹	1,550 ¹	76 ¹	18 ¹
2 weeks after.....	12,100 ¹	10,400 ¹	1,330 ¹	86 ¹	11 ¹
3 weeks after.....	7,200	6,400	575	89	8
4 weeks after.....	11,000	9,450	1,320	86	12
5½ weeks after.....	9,100	7,450	1,080	82	12
6½ weeks after.....	11,000 ¹	8,250 ¹	2,100 ¹	75 ¹	19 ¹
7 weeks after.....	8,500	7,500	510	88	6
9½ weeks after.....	Dead				

* This patient was given radium treatments at an earlier date [August 1915] in a different institution. Dosage and filtration at that time were not recorded.

(3) A readjustment of relative counts from the fourth to sixth weeks.

(4) A sharp drop in absolute and relative lymphocyte count at the seventh week without any leucocytosis, presaging the death of the patient two and one-half weeks later.

Summary. This case shows an initial depression of the lymphocytes followed by temporary recovery, and a final sharp drop shortly before death. The polymorphonuclears show no definite changes.

CASE IX	TOTAL WHITE BLOOD CELLS	TOTAL POLY- MORPHO- NUCLEARS	TOTAL LYMPHO- CYTES	PER CENT POLY- MORPHO- NUCLEARS	PER CENT LYMPHO- CYTES
Before treatment.....	8,400	5,600	1,850	67	22
1 month after.....	6,500	5,000	1,000	77	16
2 months after.....	13,000	10,300	1,700	79	13
3 months after.....	14,000 ¹	11,350 ¹	2,100 ¹	81 ¹	15 ¹
4 months after.....	15,500	14,700	450	95	3
Last count, 4 months, 9 days..	19,300	17,500	1,150	91	6
4 months, 10 days.....	Death				

Case IX. This chart shows the following:

(1) A preliminary drop in total white count, followed by a steady slight rise and slight terminal leucocytosis.

(2) A sharp drop in lymphocytes, absolute and relative, accounting for most of the preliminary drop in total white count.

(3) A steady increase in polymorphonuclear count, absolute

and relative, with corresponding decrease in lymphocytes, most marked in the relative counts.

Summary. This chart shows a marked fall in lymphocytes one month after treatment was begun, accompanied by a slight decrease in polymorphonuclear count. This is followed by a steady increase in polymorphonuclear count and an irregular lymphocytic curve. The lymphocytes do not show a further marked drop until a short time before death. The last count, taken the day before death, shows a final effort to rally on the part of the lymphocytes. The usual terminal leucocytosis is seen.

CASE X	TOTAL WHITE BLOOD- CELLS	TOTAL POLY- MORPHO- NUCLEARS	TOTAL LYMPHO- CYTES	PER CENT POLY- MORPHO- NUCLEARS	PER CENT LYMPHO- CYTES
Before treatment.....	14,000	10,800	2,250	77	16
2 weeks after.....	9,000	7,000	1,500	78	17
3 weeks after.....	9,500	6,650	2,350	70	25
4 weeks after.....	8,000	5,600	2,000	70	25
5 weeks after.....	6,500	4,200	1,800	65	28
6 weeks after.....	7,500	4,850	2,300	65	31
Last count 6½ weeks, 3 days before death.....	13,000 ¹	9,100 ¹	3,250 ¹	70 ¹	25 ¹

Case X. This chart shows a decrease in total white count from the initiation of treatment until a week before death, followed by a terminal rise. The lymphocytes show a primary drop in absolute count after the first two weeks but a steady rise in percentage count, reaching its maximum a week before death. Even after the terminal leucocytosis appears the lymphocytes maintain a high percentage, although most of the increase is in the polymorphonuclears.

Group 2

From such a mass of detailed observations it is hard to deduce any broad principle. For the sake of simplicity the remote results will again be divided into the early and late blood-changes.

Early changes. These can be studied in cases V, VIII, IX, and X.

(1) There is first to be noted, as in group 1, a primary lymphocytic drop in all cases. This is usually both absolute and relative, although in one case (X) the relative figure shows almost no change. This case is atypical in other ways, as will be seen later.

(2) Three out of the four cases (V, IX, and X) also show a fall in the absolute polymorphonuclear count, though in only one case is the fall relatively as great as the fall of lymphocytes (case X). In one case (VIII) there is no fall in the absolute polymorphonuclear count. In other words the action on the lymphocytic system is more constant and profound than the action on the polymorphonuclears.

(3) All cases show some tendency on the part of the lymphocytes to attempt a recovery; case V at nineteen weeks, case VIII at four and six and one-half weeks, case IX at three months, and case X from three to six weeks.

(4) The time at which these changes take place is not constant. The lymphocytic fall occurs at from two to four weeks and may be maintained in some degree until the end of the second month. The attempt at recovery, however, may occur at any period thereafter, from three weeks to nineteen weeks.

Late changes. (1) Terminal leucocytosis. This was found to be present in five out of six cases. The sixth case (VIII) died while no longer under observation and no autopsy was performed. It did not seem probable at the time when she left the hospital that she would die for some months, whereas she actually died two and one-half weeks later. The possibility of some intercurrent condition must be considered here, and it must also be borne in mind that she may have developed a leucocytosis after leaving the hospital.

(2) The terminal leucocytosis is due primarily to an increase in polymorphonuclears, although there is often an absolute as well as relative decrease in lymphocytes at the same time.

(3) Almost all the cases showed a tendency towards relative increase of polymorphonuclears and relative decrease of lymphocytes before the terminal leucocytosis appeared.

(4) The one case which showed no leucocytosis upon dis-

charge (case VIII), that is to say two and one-half weeks before death, did show a great decrease in absolute and relative lymphocyte counts just prior to this date, a condition which has been shown to precede this terminal leucocytosis.

(5) One case (X) showed a steady relative increase in lymphocytes from two weeks until six and one-half weeks, three days before she died. This, however, was not accompanied by an absolute increase until the terminal leucocytosis appeared. In other words, the relative increase was really due to a decrease in polymorphonuclears, in which connection it is interesting to note that the smears showed myelocytes and nucleated red cells, in spite of falling white count, indicating an exhausted bone-marrow which was endeavoring to its utmost to compensate for its loss of normal function.

From a glance at these findings it will readily be seen that the essential effects of radium treatment are the same, whether it be given in conjunction with *x*-ray or not. Thus in both groups of cases the early depressing effect on the lymphocytes, and to a less marked degree on the polymorphonuclears, is found; in both groups, also, there is a tendency to lymphocytic regeneration after varying periods in those cases whose resistance has not altogether broken down. In both groups we find a change in relative counts toward the end, the polymorphonuclears increasing, the lymphocytes decreasing; and, finally, in both groups there is almost invariably a terminal leucocytosis, due primarily to absolute increase in polymorphonuclears, but usually accompanied by some decrease in absolute lymphocyte count. It seems reasonable, therefore, to disregard the scattered *x*-ray treatments as factors in determining the blood-changes here noted.

GENERAL SUMMARY AND CONCLUSIONS

1. The blood of ten cases of squamous-cell carcinoma of the cervix uteri and vagina has been studied, in order to ascertain the immediate and remote effects of radium and *x*-ray treatments upon the formed elements of the blood.

2. The immediate effects of radium on the blood are not

altered qualitatively by previous *x*-ray or radium treatments, although the quantitative action may be somewhat diminished during a second treatment.

3. The remote effects of radium on the blood are essentially similar to the effects of combined *x*-ray and radium treatment.

4. Individual slight differences in response to radium applications are often noted to occur again on a second application in the same individual.

5. The immediate effects of radium on the blood are the following:

a. An immediate drop in total white count reaching its maximum from one-half to six hours after the application.

b. A return of the total white count to its former level within twenty-four hours after the application, usually within the first twelve hours.

c. An occasional secondary rise of the total white count to a point well above its original level from twelve hours to three days after the application.

d. A close adherence of the total polymorphonuclear count to the curve of the total white count.

e. An absence of characteristic changes in the total lymphocyte and total large mononuclear counts.

f. A tendency of the total lymphocyte count to follow in some degree the fluctuations of the total white count, especially when these are marked. This effect is not constant.

g. A tendency of the relative lymphocyte count to drop, and of the polymorphonuclears to rise during the course of treatment. This tendency is reversed during the period immediately following the removal of the radium.

6. Remote effects of radium treatment on the blood are as follows:

a. Early.

(1) Fall in lymphocyte count from two to four weeks after treatment, sometimes lasting till the end of the second month.

(2) Fall in polymorphonuclears after treatment, sometimes simultaneous with the fall in lymphocytes but usually coming later and being less striking.

(3) An attempt of the lymphocytes to recuperate, as shown by a rise in most cases at some later date, varying from three to nineteen weeks after treatment, to the approximate level seen before treatment.

b. Late.

(1) Change in the relative counts as the patient's resistance weakens, with increase in polymorphonuclears and decrease of lymphocytes, but without leucocytosis.

(2) Terminal leucocytosis, due in the main to increase of the absolute polymorphonuclear count, although usually accompanied by an absolute decrease in lymphocytes.

THEORETICAL CONSIDERATIONS

In conclusion, it may not be amiss to speculate briefly upon some points brought out in the above study.

1. In the first place, what do these figures show us to prove or disprove the theory that the lymphocyte is the essential factor in immunity or resistance to tumors? It seems fairly clear from the above that in the terminal stages of death from cancer there is a relative and often an absolute lymphopenia. It is, however, also true that this lymphopenia may not appear until a few days before death (case X); in fact, there may be a slight excess of lymphocytes as late as this. It would hardly seem reasonable, therefore, to assume that the lymphocyte is an infallible index of the patient's state of resistance at any given period, seeing that the patient in question (case X) was just as surely moribund one week before death, when she had 31 per cent lymphocytes and a total lymphocyte count of 2300, as she was four days later when the percentage had dropped to 25. It would then be useless to attempt to estimate a patient's resistance at any given time by referring to the absolute and relative lymphocyte count. On the other hand, if the blood has been systematically followed over a long period, it is possible to predict a fatal ending by the changing relative counts of lymphocytes and polymorphonuclears, the former decreasing, the latter increasing, though the practical value of this fact is very small, inasmuch

as the same conclusion may be reached at an earlier date by clinical observation.

2. Can these changes in the blood be used as a guide to the proper intervals and dosage of radium? This question can not be answered on the basis of the above figures, inasmuch as all the patients were so far advanced in the disease as to be beyond the hope of cure, at least with the small amount of radium which we had at our command.

Seeing, however, that the first sign of the final collapse is given by a fall in relative lymphocyte count, it does not seem unreasonable to suggest that no second radium treatment should be given until the lymphocyte count has recovered from the preliminary depression consequent to the first application. It would be interesting to know the behavior of the blood in patients treated under more favorable circumstances, that is to say, with larger doses and few applications.

3. Do these figures indicate anything of importance in regard to the mode of action of radium? It is certainly striking that the response of the hematopoietic organs to the application of so small an amount should be so immediate and yet so lasting. If we are to accept the theory that the beneficial effects of radium are dependent solely upon its local action, it must first be proved that these profound blood-changes are not incompatible with complete and lasting cure. In one instance we have known of a case which had been treated with heavy dosage of radium coming to autopsy without showing any demonstrable remains of carcinoma or any morbid process that could explain death. No studies of the blood or blood-forming organs were made. Is it not conceivable that in this case, although the carcinoma was locally cured, the patient was killed by the radium? The authors have been particularly interested in the close analogy between the immediate blood changes following radium treatment, as noted above, and those mentioned by Jobling (11) and Scully (12) as occurring after the intravenous injection of foreign protein in the treatment of arthritis and other conditions. Similar observations have been made by our colleague, Dr. B. F. Schreiner (13), following injections of

phylacogen. It is not impossible that the early blood-changes following radium application are due to the sudden introduction of protein substances, the products of cell destruction, into the blood stream, with the same effects on the blood-forming organs as are brought about by the intravenous injection of foreign protein. Even if this be the case, however, we are not justified in assuming that the therapeutic effect of radium is dependent on the formation of these products of abnormal cell destruction. It should be emphasized, in closing, that, while we have abundant evidence, both clinical and histological, that radium has a definite local action on malignant growths and that this action is detrimental to their development, there is also evidence of a more general effect whose nature we do not by any means understand. It is to be hoped that further blood-studies from the physicochemical as well as from the morphological standpoint will clear up this obscurity, and will enable us to explain some of the miraculous cures and unexpected disappointments which we encounter when employing radium as a therapeutic agent.

Finally, we wish to express our indebtedness to Dr. H. R. Gaylord, Director of the Institute, for valuable suggestions at the outset of the work, and to Miss Alice Thornton for her careful attention to the details of the routine counts and charts

REFERENCES

- (1) GUDZENT, F.: *Strahlentherapie*, 1913, ii, 467.
- (2) HEINEKE, H.: *Mitteil. a. d. Grenzgeb. d. Med. u. Chir.*, 1904, xiv, 21.
HARVEY, W. G.: *Jour. Path. and Bacteriol.*, 1908, xii, 549.
- (3) MILCHNER AND MOSSE: *Berl. klin. Wehnschr.*, 1904, li, 1267.
HELBER AND LINSE: *München. med. Wehnschr.*, 1905, li, 689.
- (4) AUBERTIN AND BEAUJARD: *Compt. rend. Soc. de biol.*, 1905, lviii, 217.
- (5) MORRIS, R. S.: *Am. Med.*, 1905, x, 946.
- (6) ALLEN, S. W.: *Jour. Med. Research*, 1903, ix, 467.
- (7) KOLDE, W., AND MARTENS, E.: *Strahlentherapie*, 1915, v, 127.
- (8) SCHWEITZER, B.: *München. med. Wehnschr.*, 1916, lxiii, 341.
- (9) PAPPENHEIM: *Atlas der menschlichen Blutzellen*, ii; Jena, 1905.
- (10) WERNER, R.: *Beitr. z. klin. Chir.*, 1906, lii, 51.
- (11) JOBLING, J. W.: *Arch. Int. Med.*, 1917, xix, 1042.
- (12) SCULLY, F. J.: *Jour. Am. Med. Assn.*, 1917, lxix, 20.
- (13) SCHREINER, B. F.: Personal communication.

PROTOCOLS OF PATIENTS WITH CHARTS ILLUSTRATING IMMEDIATE AND REMOTE EFFECTS OF RADIUM APPLICATIONS

Case I. L. S. Married. Age 48. Metrorrhagia, June, 1915. Percy cautery operation, July, 1915. Hemorrhage (approximately 500 cc.) three weeks later. Entered hospital June, 1915. Ulcerated immobile cervix. Died November, 1916. Multiple subcutaneous metastases. Fifteen radium applications; sixteen x-ray treatments. (See chart 20.)

Case II. M. P. Single. Age 30. Bloody vaginal discharge, December, 1914. Hysterectomy, January, 1915. Gained 30 pounds, but bladder very irritable. Bleeding recurred February, 1916. Entered hospital at this time. Nodular immovable mass in vault of vagina. Urinary incontinence, August, 1916. Sciatica, October, 1916. Died, November, 1916. Metastases in retroperitoneal lymph nodes; invasion of bladder and sciatic nerve. Eight radium and eleven x-ray treatments. (See chart 21.)

Case III. M. K. Married. Age 30. Menorrhagia, 1912-15, increasing and later developing into continuous bleeding. Curettage, August, 1915. Hysterectomy, September, 1915. Discharge and bleeding, March, 1916. Entered hospital at this time. Immovable, indurated, ulcerated mass in vaginal vault. Foul watery discharge. Recto-vaginal fistula, August, 1916. Died, December, 1916. No autopsy. Three radium treatments. (See chart 22.)

Case IV. M. H. Married. Age 40. Bloody vaginal discharge, November, 1915. Hemorrhage, March, 1916. Cautery applied. Entered hospital, April, 1916. Large ulcerated, hard tumor involving cervix and left broad ligament. Cervix immovable. Hemorrhage, July, 1916. Uraemia and death, August, 1916. Metastases in lungs; chronic nephritis. Two radium treatments. (See chart 23.)

Case V. E. W. Married. Age 50. Menorrhagia, 1913. Curettage. Bleeding returned three months later. Sudden anuria, April, 1916. Cystotomy, August, 1916. Admitted to hospital two weeks later. Extensive induration along anterior vaginal wall and cervix. Urinary flow returned after first radium treatment. Metastatic lymph nodes in groin removed, December, 1916. Uraemia and death, February, 1917. Metastases in retroperitoneal lymph nodes, liver, and lungs; invasion of bladder and rectum. Double hydronephrosis. Seven radium and seven x-ray treatments. (See chart 24.)

Case VI. M. W. Widow. Age 56. Hemorrhages, June, 1916 and

August, 1916. Admitted, September, 1916. Large spongy mass springing from cervix and invading right broad ligament. Marked secondary anaemia. Gradual loss of strength. Died, January, 1917. Metastases in peritoneum and lung; invasion of bladder; chronic nephritis. Five radium treatments. (See chart 25.)

Case VII. P. S. Married. Age 42. Mass in uterus, 1912. Specimen removed; said not to be cancer. Bleeding by vagina, 1914. Panhysterectomy, 1914. Watery discharge for past two weeks. Admitted to hospital, September, 1916. Ulcerating nodular mass in vault of vagina. Bloody discharge. Vesico-vaginal fistula, November, 1916. Has not reported since. Three radium treatments. (See chart 26.)

Case VIII. J. F. Married. Age 59. Polyp removed from cervix, June, 1915. Cautery applied, August, 1915. Bloody vaginal discharge continued. Radium treatment for supposed fibroids, September, 1915. Severe external and internal burns resulted. Vaginal discharge continued, often bloody. Nodules recently discovered in vaginal wall by physician. Severe pain in lower abdomen. Admitted to hospital, October, 1916. Uterus enlarged. Many small nodules in vaginal wall. Tenderness beneath scar of old burn in right lower quadrant. Small metastases in both groins. Gradual loss of weight. Discharged December, 1916. Died one week later. No autopsy. Three radium and one x-ray treatment. (See chart 27.)

Case IX. C. M. Widow. Age 50. Painful urination, spring of 1916. Occasional hemorrhages since July, 1916. Watery vaginal discharge for past six weeks. Admitted to hospital, November, 1916. Extensive nodular induration of entire vaginal wall. Exquisite tenderness, making examination almost impossible. Temporary local improvement, followed by rapid loss of weight and strength. Died, March, 1917. Autopsy: Extensive metastases in reoperitoneal lymph nodes, lungs, and liver; invasion of bladder. Two radium and four x-ray treatments. (See chart 28.)

Case X. B. C. Married. Age 43. Menorrhagia, 1913. Curettage. Diagnosis of cancer made. Panhysterectomy, March, 1914. Well until three months ago. Since then severe pains in hips, back, and shoulders. Bed-ridden. Small ulcer in vaginal vault. Large masses in both iliac fossae. Severe sweats. Oliguria. Loss of pain under treatment and decrease in size of masses. Gradual loss of weight and strength; death in uraemia, January, 1917. Autopsy: chronic nephritis; bronchopneumonia; metastases in retroperitoneal

lymph nodes, lungs, and liver; meningitis. Local lesion almost destroyed. (See chart 29.)

Case XI (control). L. Z. Married. Age 35. Vaginal discharge, sometimes bloody, since March, 1916. Hemorrhage, March, 1917, followed by slight continuous bleeding. Admitted to hospital, May, 1917. Examination showed extensive ulcerated, nodular mass, involving cervix and broad ligaments. Considerable local and general improvement under treatment. Two radium treatments. Patient still in the hospital. (See chart 30.)

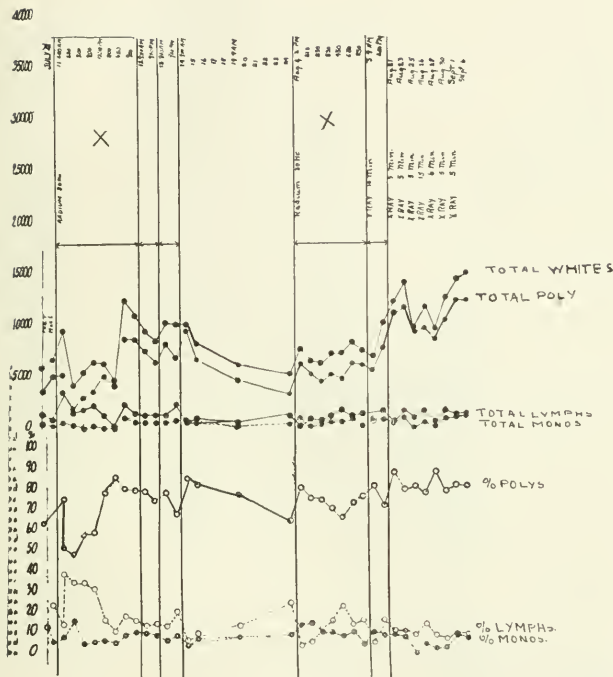


CHART 20

Days on which more than one count was made are designated by cross-lines with arrow-points at either end. The heavy vertical lines at either end of the cross-lines represent the beginning and end of the day in question. Except where marked by these cross-lines only one count was made on any given day. Days chosen for studying the immediate blood changes are marked thus: x.

1. Primary drop in lymphocytes, followed by temporary recovery, and final relative decline.

2. Diminished response to second radium treatment.

3. Terminal leucocytosis.

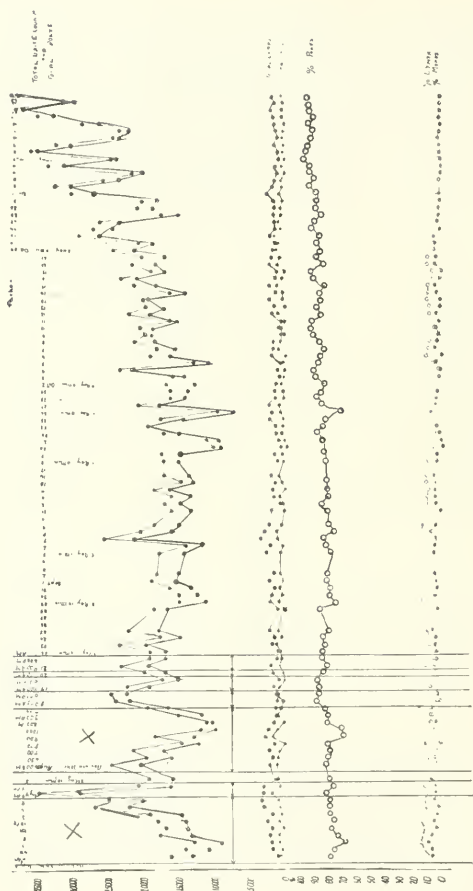


CHART 21

For explanation of vertical and cross-lines see under case 1.

1. Steady decline in total lymphocyte count.
2. Terminal leucocytosis, preceded by decrease in relative lymphocyte count and increase in relative polymorphonuclear count.
3. Typical immediate blood-changes following radium application, somewhat modified on second application.

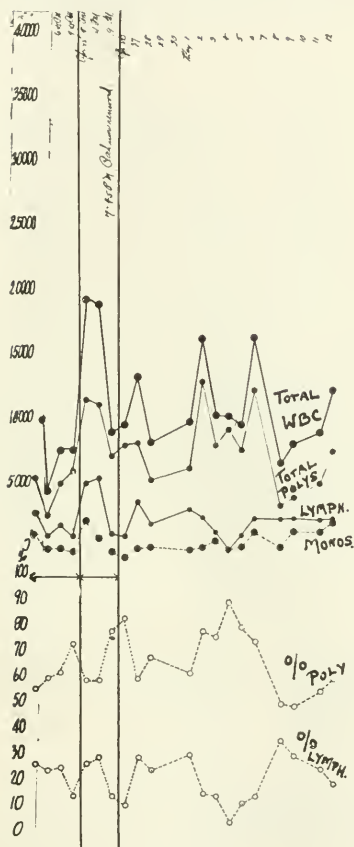


CHART 22

For explanation of vertical and cross-lines see under case 1.

1. Typical early reaction, with immediate drop of white count, followed by secondary rise.
2. Primary drop in lymphocytes reaching lowest point at end of fifth week, followed by recovery.
3. Slight depression of polymorphonuclear series at end of sixth week.

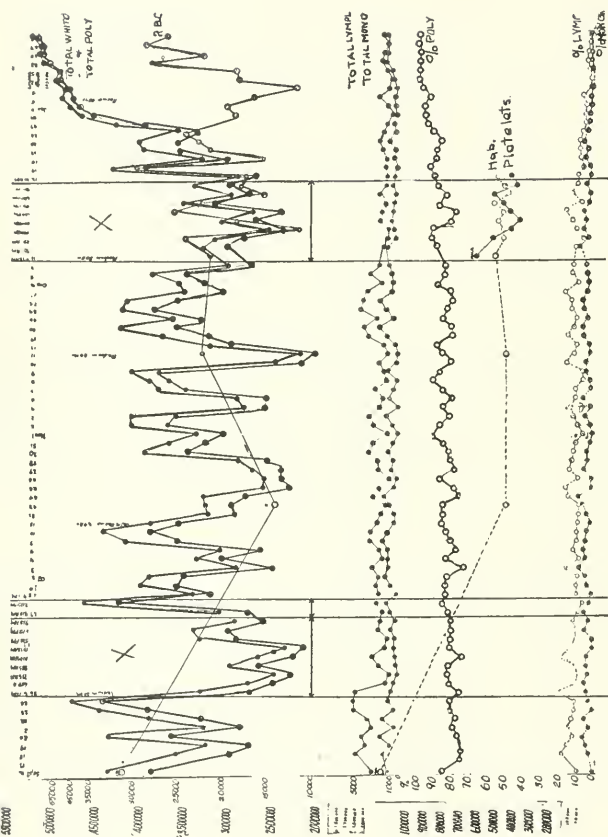


CHART 25

For explanation of vertical and cross-lines see under case 1.

1. Characteristic reaction in white cells immediately after radium application, less pronounced the second time than the first.
2. Tendency of total lymphocytes to fall from the time of the first application.
3. Irregular curve of total polymorphonuclears, with tendency to fall.
4. Marked terminal polymorphonuclear leucocytosis, with consequent drop in relative lymphocyte value.
5. Immediate drop in platelet and red cell counts, followed in the case of the red cells by a compensatory rise and, later, by a second drop. The red cells show a terminal rise, corresponding to the rise in polymorphonuclears.

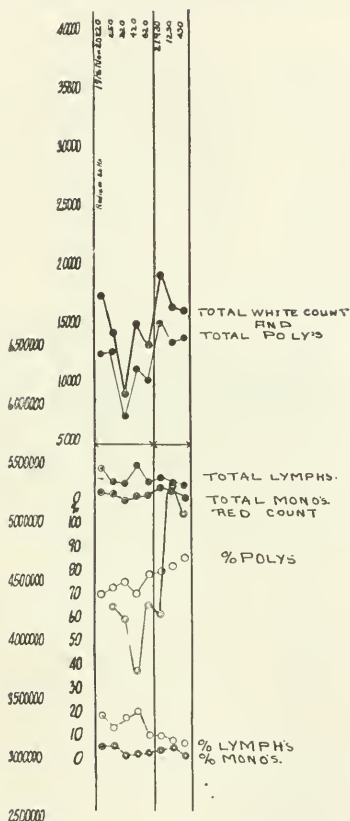


CHART 26

For explanation of vertical and cross-lines see under case 1.

1. Characteristic primary drop in white count.
2. Change in percentage values of lymphocytes and polymorphonuclear cells during treatment.
3. Drop in red cell count, followed by marked rise.

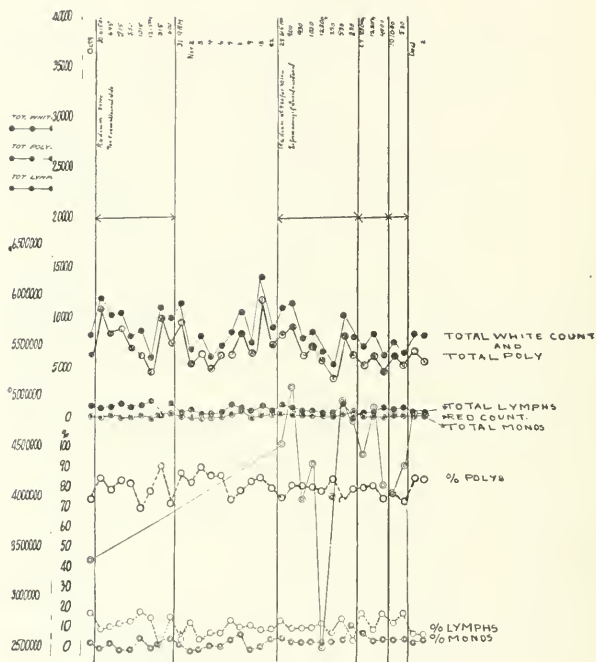


CHART 27

For explanation of vertical and cross-lines see under case 1.

1. Characteristic immediate drop in white count.
2. Absence of secondary rise.
3. Preliminary slight drop in lymphocytes, followed by recovery.
4. Fall in lymphocytes just before discharge, both in total numbers and percentage value, indicating near approach of terminal leucoeytosis. The patient left the hospital before any leucoeytosis developed, but died less than three weeks later.

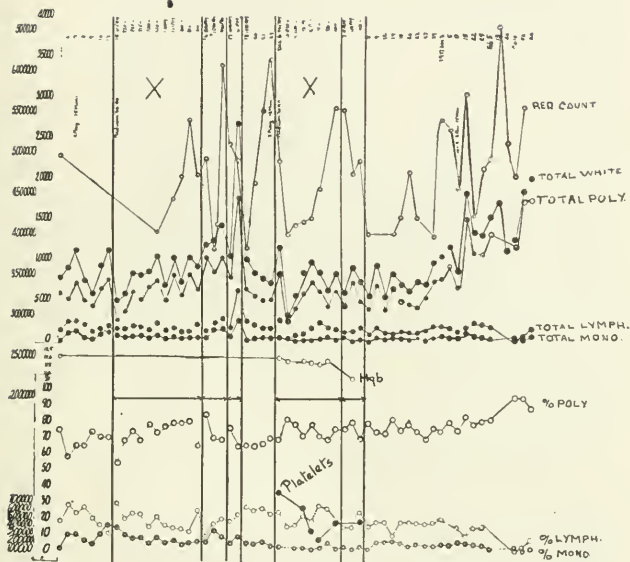


CHART 2S

For explanation of vertical and cross-lines see under case 1.

1. Absence of secondary rise of white count after radium application. The usual primary drop is seen.

2. Tendency for slight decrease in total and percentage counts of lymphocytes.

3. Tendency to slight drop in total polymorphonuclear count, followed by terminal leucocytosis.

4. Immediate drop in platelets during radium application.

5. Drop in red count during early part of radium treatments, followed by an early secondary rise, and later by a second drop.

6. The red cells show a terminal rise, synchronous with the terminal leucocytosis.

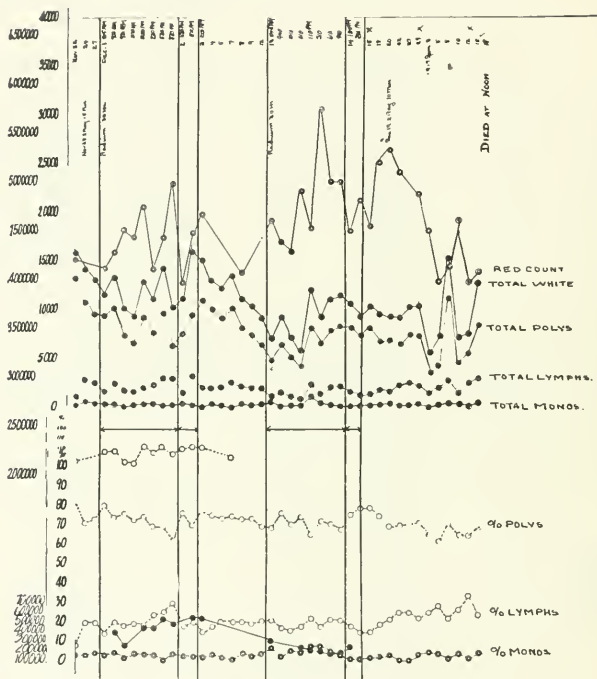


CHART 29

For explanation of vertical and cross-lines see under case 1.

1. Atypical immediate changes in white count: the same preliminary rise noted in subsequent treatment.
2. Steady drop in polymorphonuclears until just before the terminal leucocytosis appears.
3. Preliminary slight drop in total lymphocytes, corresponding to the drop in polymorphonuclears; this is followed by a steady rise until a few days before death. The rise in lymphocytes accounts for part of the slight terminal leucocytosis.
4. Rise of red count during each application, followed by return to a point below the former level.

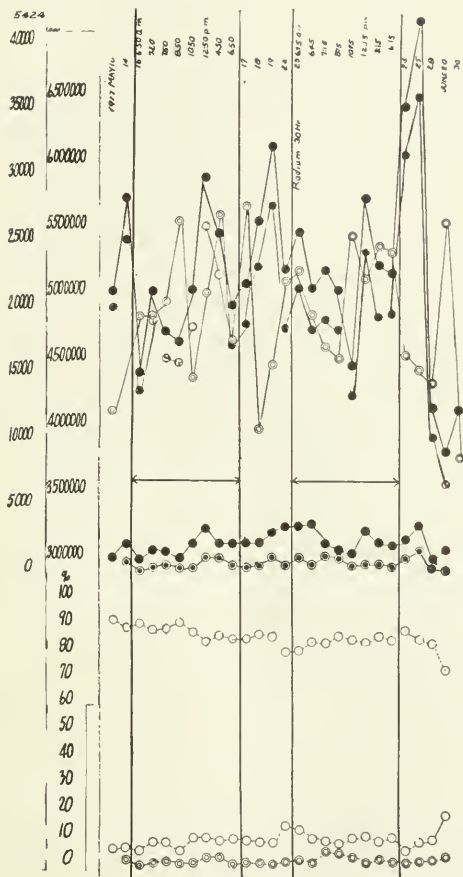


CHART 30

For explanation of vertical and cross-lines see under case 1.

1. Absence of preliminary drop in white count during control period; marked drop during treatment.

2. Absence of secondary rise in white count during control period; marked rise on second day after radium application.

THE INFLUENCE OF HEAT AND RADIUM UPON INDUCED IMMUNITY AGAINST TRANS- PLANTED ANIMAL TUMORS

G. L. ROHDENBURG AND F. D. BULLOCK

*From Columbia University, George Crocker Special Research Fund, F. C. Wood,
Director*

Received for publication, August 13, 1917

In several publications, Bashford (1) has called attention to the relationship existing between the growth energy of transplanted tumors and their power to induce the resistant state, and has also correlated the growth energy of these tumors with their resistance to the immunizing action of living cells. Broadly stated, his view point is: that the power to overcome the resistant state varies directly as the growth energy of the tumor, whether immunity result from the growth of the tumor itself (concomitant immunity) or be caused by normal living cells.

If Bashford's view be the correct one, then it should be possible to immunize against tumors which are refractory to the induced resistant state, and to increase the percentage of induced immunity against susceptible tumors, by depressing the growth energy of these neoplasms. This has, in fact, been done by Tsurumi, who (2) has demonstrated that certain sarcomata which are resistant to induced immunity can be rendered susceptible by exposing them to comparatively low degrees of heat for varying periods before inoculation. It should also be possible to bring out more distinctly the concomitant immunity produced by various growing tumors.

The growth energy of transplanted tumors can be depressed, also, by radium, as Wedd and Russ (3), and Wood and Prime (4), have shown. The retardation of growth energy was not a permanent alteration in the biology of the tumor, however, and persisted only for a few generations of transplants.

With these facts in mind, a series of observations upon the influence of radium and heat in induced immunity was undertaken with various tumor strains. The strains chosen were the Crocker Fund mouse sarcoma 180 and the Ehrlich mouse sarcoma, both refractory to the immunizing power of living cells, and the Jensen rat sarcoma, which is susceptible to the induced resistant state.

The technic used in depressing the growth energy of the tumors was as follows: 83 mgm. of radium element, screened by 0.4 mm. of brass and a thin glass cover-slip, were placed upon a strip of tumor 2 mm. thick, 2 to 3 mm. wide, and 20 mm. long, so that the screened radium capsule was superimposed upon the tumor material, strict aseptic precautions being observed. The exposure to radium was continued for thirty minutes.

When heat was used, strips of tumor of dimensions similar to those of the strips exposed to radium were placed in Ringer's solution in the incubator at 38°C. for two hours.

Portions of the tumor strain to be used in a given series were treated by radium or heat to depress their growth energy, according to the technic just described. Inoculations by needle of 0.003 gram¹ of the treated tumor were made into one group of animals immunized ten days previously with 0.05 cc. of spleen emulsion, and into another series of animals not immunized. These two series gave the percentage of induced and natural immunity with treated tumor. Another group of animals immunized ten days previously with spleen emulsion, in doses of 0.05 cc., were inoculated with 0.003 gram of the untreated tumor by needle. A final group of nonimmunized control animals was inoculated with similar doses of the untreated tumor at the same time. The last two groups inoculated with untreated tumor served to show the percentages of natural and induced immunity for the untreated neoplasm.

¹ In previous publications from the Imperial Cancer Research Fund and from this laboratory, the inoculation dose, when the needle method is used, has been estimated as 0.01 or 0.02 gram; but such grafts have recently been found, as a matter of fact, to weigh about 0.002 and 0.003 gram respectively.

In order to control the influence of the immunizing agent, the experiment was repeated with a fourth tumor strain, the English mouse carcinoma 199, a neoplasm which is moderately resistant to the immunizing power of living cells (Russell, (5)).² The technic and method used with this strain differed from that previously described only in that fetal skin emulsion was used instead of splenic pulp.

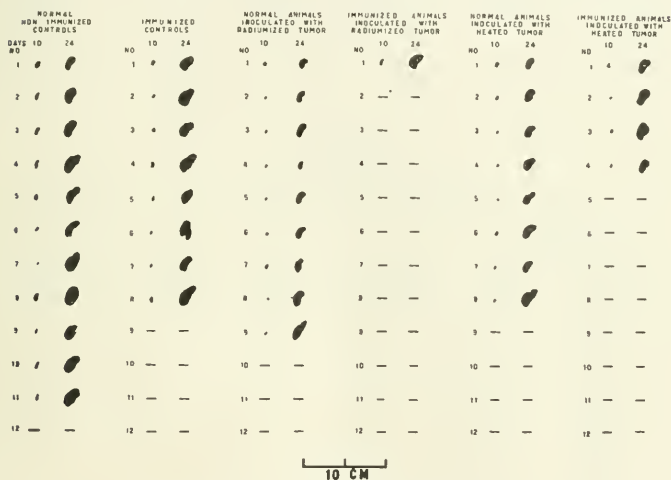


CHART 1. JENSEN RAT SARCOMA

Comparative influence of radium and heat previous to inoculation into immunized animals.

Charts 1, 2, 3, and 4 graphically portray our results in single unselected lots of 12 animals each, the entire material not being presented in chart form because of the large number of animals used.

Table 1, which gives the numerical data of each experiment,

² This laboratory is indebted for the tumor strain in question to Dr. J. A. Murray, Director of the Imperial Cancer Research Fund of London.



10 CM

CHART 2. MOUSE CARCINOMA 199

Comparative influence of radium and heat previous to inoculation into immunized animals.



10 CM

CHART 3. MOUSE SARCOMA 180

Comparative influence of radium and heat previous to inoculation into immunized animals.

shows that depression of the growth energy of the four tumor strains chosen, whether caused by radium or heat, results in a marked increase in their susceptibility to the immunity produced by splenic pulp. The percentage of induced immunity against untreated tumor, as averaged for all four strains, was 29.25, while the groups immunized and inoculated with radiumized

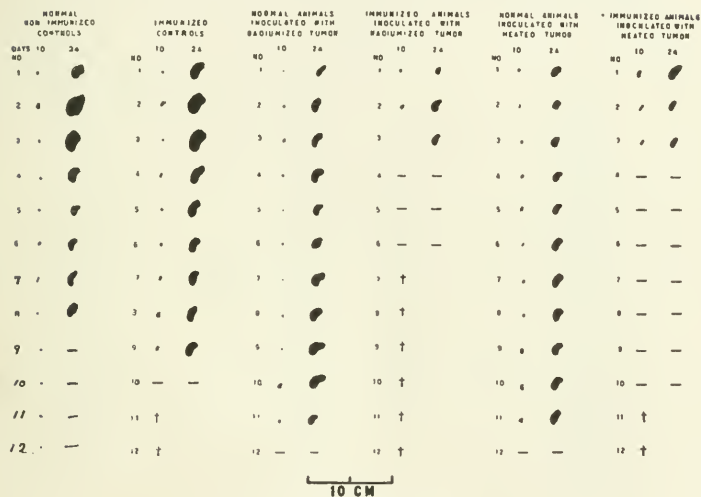


CHART 4. EHRLICH MOUSE SARCOMA

Comparative influence of radium and heat previous to inoculation into immunized animals.

tumor showed an average of 62.75 per cent and those immunized and inoculated with heated tumor showed 81 per cent average immunity, an increase of over 100 per cent in both treated groups. As is shown in both the table and the charts, our results were not influenced by the type of immunizing material used. It will also be noted in the charts that the growth energy of the treated tumors was depressed, their size on a given day being smaller than the untreated neoplasms.

The fact having been verified that depression of growth energy renders tumors more susceptible to the immunizing power of homologous living normal cells, it was determined to investigate the influence of depression of growth energy upon other phases of immunity.

In the succeeding experiments radium was used to depress the growth energy.

TABLE I

		TUMOR STRAIN							
		180		199		Ehrlich sarcoma		Jensen sarcoma	
		+	-	+	-	+	-	+	-
Normal controls inoculated with normal tumor	Number	88	1	20	7	16	10	18	1
	Per cent	99	1	74	26	61	39	95	5
Immunized controls inoculated with normal tumor	Number	60	16	13	9	9	1	14	11
	Per cent	78	22	59	41	90	10	56	44
Normal animals inoculated with radiumized tumor	Number	90	2	16	3	20	1	18	6
	Per cent	98	2	84	16	94	6	75	25
Immunized animals inoculated with radiumized tumor	Number	37	35	8	38	3	3	2	18
	Per cent	51	49	18	82	50	50	10	90
Normal animals inoculated with heated tumor	Number	17	2	17	6	21	1	13	10
	Per cent	89	11	73	27	95	5	57	43
Immunized animals inoculated with heated tumor	Number	4	8	0	14	3	7	8	16
	Per cent	12	88	0	100	30	70	34	66

+ = takes.

- = no take.

Number = actual number of animals used.

Per cent = percentage of takes or immunity as the case may be.

That the resistance of a graft to concomitant immunity is decreased by radium is shown in the following experiment. Thirty-three rats bearing eighteen day old Flexner-Jobling carcinomata were divided into two groups. This tumor under ordinary conditions does not produce concomitant immunity. One group of eighteen animals received a second inoculation of 0.003 gram of the same tumor strain, the inoculated material having

been exposed to radium in the manner described in a previous paragraph. The second group of fifteen animals was inoculated with a similar dose of the same tumor not exposed to radium. A control group of ten normal animals was also inoculated with radiumized tumor.

The results, as depicted in chart 5, show 44 per cent concomitant immunity in the group inoculated with radiumized tumor,

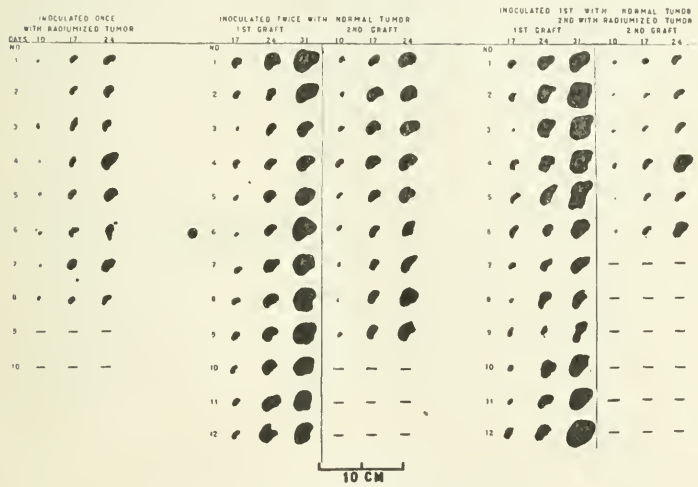


CHART 5. FLEXNER-JOBLING RAT CARCINOMA

Effect upon concomitant immunity of radiating second graft

as compared with 20 per cent concomitant immunity in the group inoculated with normal tumor. The normal animals inoculated with radiumized tumor also exhibited 20 per cent natural immunity, showing that radiation had not killed the tumor.

The influence of radium upon the processes of induced immunity was studied in still another experiment. A series of rats was inoculated with the usual dose of the Flexner-Jobling

tumor. Five days after inoculation, the stroma reaction being established, the skin over each graft was exposed for five minutes to 83 mgm. of radium element, unscreened. Upon the same day one-half of this group (36 rats) was injected with 0.05 cc. of rat spleen emulsion, the other half of the group serving as controls. The animals were immunized on the day of

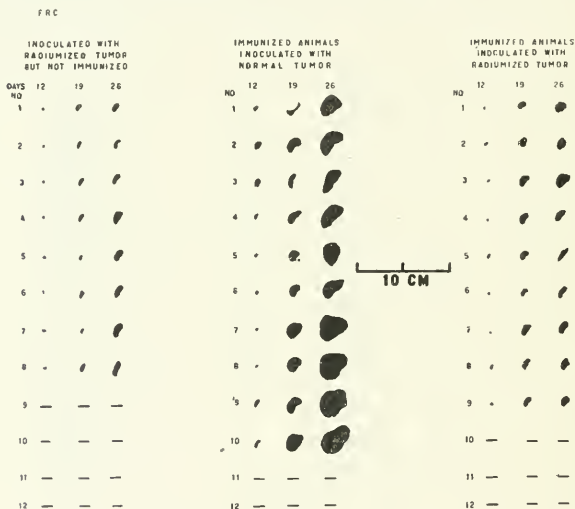


CHART 6. FLEXNER-JOBLING RAT CARCINOMA

Attempt to induce immunity after inoculation with radiumized tumor

radiation so that both radium and immunity might exert their maximum effect simultaneously, i.e., about the tenth day.

A further control group of 36 animals which had been inoculated at the same time as the other two groups was also immunized with 0.05 cc. of rat spleen pulp.

As is shown in chart 6, twenty-six days after radiation the tumors had disappeared in 25 per cent of those radiated and immunized, as compared with 27 per cent in those radiated but

not immunized. As the chart shows, the two groups with radiated tumors show inhibition of tumor growth as compared with the nonradiated animals, though radiation had not been sufficient to kill the tumor completely. A tumor having once been established, therefore, the retardation of its growth energy by radium does not make it any more susceptible to immunity than are growing tumors that have not been radiumized, a result in complete accord with the previous work of Russell (5), who used untreated tumor. As the established tumor which has been radiumized is not subject to immunity reactions, it is much less probable that the tumor which has not been radiumized is in any way sensitive to immunity reactions. This obser-

TABLE 2

	NO. OF ANIMALS	PERCENT- AGE OF IMMUNITY
Normal animals with normal tumor.....	19	0
Normal animals inoculated with radiumized tumor.....	20	5
Immunized with mouse carcinoma, inoculated with normal tumor.....	20	10
Immunized with mouse carcinoma, inoculated with radiumized tumor.....	33	9
Immunized with autologous skin, inoculated with normal tumor.....	15	10
Immunized with autologous skin, inoculated with radiumized tumor.....	12	0

vation, therefore, casts doubt upon the power of radium or x -ray to affect metastases of tumors at a distance from the growth radiated, as has been repeatedly claimed by clinical workers. Clinicians have stated that the radiumization of a primary breast tumor causes nodules in the axilla to disappear, and have proposed the hypothesis that ferments are set free from the primary tumor under the influence of the radiumization which act in an unfavorable manner upon masses at a distance from the primary growth. The experiments detailed above, however, show that the shrinkage and disappearance of such nodules—if they really do disappear—must be due to some other influence than the hypothetical ferment. The very great suscepti-

bility of lymphoid tissue to even slight radiation is well known, and any change in the size of the lymph-nodes, even if they are invaded by carcinoma, may be due to reduction of inflammatory edema and to the destruction of the lymphoid elements, thus leaving the carcinoma untouched. This latter phenomenon has been repeatedly observed where clinical cures have been obtained of carcinomatous nodules, a marked shrinkage taking place upon relatively slight radiation. If such nodes are excised and examined, it is found that the lymphoid tissue has disappeared, but that the carcinoma, which forms only a small portion of the total mass, has remained as active in its growth as ever; and, in fact, those cases which have been followed have shown that ultimately recurrence takes place, even though the nodule after radiation was greatly reduced in size.

In a final experiment, the possibility of making tumors susceptible to attempts to immunize with heterologous and autologous tissue was investigated. Although some investigators have described the appearance of immunity after the introduction of heterologous tissues, the majority have not succeeded in confirming their results; and almost all are agreed that autologous tissues are powerless to confer immunity.

The Flexner-Jobling rat carcinoma was the tumor chosen for this experiment, the radiation, the inoculation dosage, and the methods being the same as those described in the early paragraphs of this paper. The animals were divided into several groups, the rats of the first series being immunized with 0.05 cc. of skin taken from the animals themselves, those of the second series with 0.05 cc. of mouse carcinoma, while those of the third series were not immunized. Ten days after immunization, one-half of the animals of each series were inoculated with untreated tumor, the other half receiving inoculations of radium-ized tumor. Thirty-one days after inoculation, our results showed that exposure to radium did not make the Flexner-Jobling carcinoma susceptible to either autologous or heterologous tissues. The numerical data of the experiments are given in table 2.

CONCLUSIONS

Depression of the growth energy of a transplantable tumor by heat or by exposure to radium increases its susceptibility to the immunizing action of homologous living cells, whether they be normal or tumor cells. With the technic as described in this paper the additional immunity thus obtained may be 100 per cent over the usual figure.

The increased susceptibility of treated tumor to the immunizing power of living cells is not in evidence when autologous elements are employed, or when homologous tissues are introduced subsequent to tumor implantation.

Tumors which have established a residence in their hosts, i.e., have excited a stroma reaction and obtained a blood supply, are not influenced by retardation of their growth energy with radium and the simultaneous introduction of homologous living cells.

Thus, none of these results leads in any way to the conclusion that the improvement described in human cancer under radium treatment is due to the stimulation of autologous cells or that benefit is to be expected by radiumization and previous or subsequent injection of homologous lymphoid tissue. Even though the tumors employed in these experiments had been cured, the results could not have been transferred to man, where the problem is to cure a spontaneous (as distinguished from a transplanted) neoplasm. As an animal can not be immunized against its own tumor, or with its own tissues, it is readily seen that the application of such experiments as these to man can hardly lead to a profitable result.

REFERENCES

- (1) BASHFORD, E. F., MURRAY, J. A., AND CRAMER, W.: Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 315.
- (2) TSURUMI, I.: Jour. Path. and Bacteriol., 1915, xx, 76.
- (3) WEDD, B. H. AND RUSS, S.: Jour. Path. and Bacteriol., 1912-13, xvii, 1.
- (4) WOOD, F. C. AND PRIME, FREDERICK: Ann. Surg., 1915, lxii, 751.
- (5) RUSSELL, B. R. G.: Fifth Sci. Report, Imperial Cancer Research Fund, London, 1912, 1.

FLUCTUATIONS IN THE GROWTH ENERGY OF MALIGNANT TUMORS IN MAN, WITH ESPECIAL REFERENCE TO SPONTANEOUS RECESSION

G. L. ROHDENBURG

*From Columbia University, George Crocker Special Research Fund, F. C. Wood,
Director*

Received for publication, August 14, 1917

It is a generally accepted fact that transplanted animal tumors may fluctuate widely in their growth energy, the fluctuations varying from a temporary cessation of growth followed by renewed cellular activity to recession and complete disappearance of the tumor. Hence, warning has repeatedly been given against drawing deductions from the results of experimental therapeutic procedures in animals without proper consideration of these natural changes in the growth energy of tumors.

Though this is not widely known, the warning is applicable also to the spontaneous tumors of man, since fluctuations of a similar character are a not uncommon occurrence in them, as the reviews of this subject by Lomer, Mohr, Gaylord and Clowes, and others, amply demonstrate. Handley has well expressed the situation as follows:

The progress of a cancer is normally accompanied by regressive or curative processes. The recorded cases of natural repair of cancer, far from being anomalous and exceptional, merely illustrate more strikingly than usual the natural laws which govern every case of the disease.

Indeed, the literature of cancer is rich in reports of the recession of malignant tumors after one or another type of medical treatment, the particular therapeutic procedure which is supposed to have brought about the recession in each case being at once hailed as a "cure," only to give rise to failure and disap-

pointment when tried out on a large group of cases. It is probable, therefore, that the isolated cases referred to are really spontaneous recessions and not therapeutic cures.

With the idea of emphasizing the frequency of variations in the growth energy of human tumors, and at the same time of seeking further facts bearing upon these variations in proliferative activity, the cases of marked recession or spontaneous cure recorded in the literature have been summarized, additional cases added, and the entire material subjected to analysis from the standpoint of our present knowledge of experimental cancer.

While numerous interesting, and possibly authentic, cases of recession of human malignant tumors are recorded previous to 1890 (2), many of these have been eliminated from the present survey because the correctness of the diagnosis is open to very grave question. The factor of diagnostic error in this connection was recognized as far back as 1852 (3).

A criticism affecting many of the reported cases is the absence of any statement of an histological examination. The importance of such an examination is clearly shown by Czerny, who has reported an instance of the spontaneous disappearance of a supposed sarcoma of the femur, which upon more detailed examination was shown to be chronic osteomyelitis, due to staphylococcus infection. While in some of the reports it is evident from the general context that an histological examination has been made, all that is definitely stated is some such inexact diagnosis as "undoubtedly cancerous," or "malignant."

The lack of histological examination emphasizes errors in diagnosis, particularly in those cases where syphilis is strongly to be suspected. Thus many of the older reports have to do with primary tumors of the liver; primary malignant tumors of this organ are comparatively rare, however, and it is more than probable that these reported tumors were gummata. Again, the much more recent surgical experience with syphilis of the stomach and its confusion with carcinoma, shows that the factor of error in diagnosis still warrants very serious consideration.

In the present review, cases obviously open to question regarding the correctness of the diagnosis have been omitted,

except when the case has been quoted by some one other than the original author. When others have considered the case as bona fide, it has been quoted, and the reason for disagreement given.

That different neoplasms vary in their growth energy is constantly recognized by all clinicians; thus it is well known that, as a class, basal-cell epitheliomata grow very slowly, while gastric carcinomata have a high rate of proliferative activity. The influence of age and of certain physiological states, like lactation and pregnancy, upon the rate of tumor growth are also well recognized, youth, pregnancy, and lactation favoring a high degree of proliferative activity. With the exception of advanced age, there are no clearly recognized conditions which tend to inhibit the growth of a neoplasm.

* It can be definitely asserted that regressive changes varying from a temporary standstill to the complete disappearance of the tumor, whether it be of epithelial or connective tissue origin, may occur at any age period, in either sex, and irrespective of the location of the growth. * Though no accurate data can be given for the frequency of complete regression, Bashford has estimated that it occurs about once in a hundred thousand cases.

* There are several types of recession: so, for example, various neoplastic deposits may cease growing for a time and actually become to some degree smaller, only to assume their proliferation again at a later period; or any or all of the new growths may be completely absorbed and the patient be apparently cured. *

The clinical conditions which have been associated with regression have been various, though it has become evident, at any rate, that the forces at work may be either local or general. In those instances where only certain groups of neoplastic cells recede, while others continue growing, the recession is best accounted for on the basis of some local tissue reaction. In others, however, where all the neoplastic deposits are absorbed, some reaction taking place throughout the entire organism offers the best explanation. Nevertheless, there has been so far no demonstration of any antibody or ferment in the body which might be assumed to effect such a result.

The local conditions associated with the recession of neoplastic deposits are recognizable for the most part only by microscopic examination. These histological changes have been thoroughly studied by many observers, though unfortunately, as is the case with much of the histological investigation of cancer, the problem has not been solved. Like other tissues, tumors may be the site of any of the various types of infection or degeneration, which may result in the death and subsequent absorption of the particular group of cells affected, though other neoplastic cells in the neighborhood continue to grow.

We shall not enter into a discussion of the various types of degeneration which may be followed by recession, except to note that necrosis followed by calcification is the type most frequently associated with spontaneous absorption. Degeneration or infection having occurred, the affected cells die and are removed by the body forces which are at work when similar changes occur in nonmalignant tissues. The defect is repaired by granulation tissue.

Where neither degenerative changes nor infection can be determined as having initiated healing, the process is slightly different. Several human cases have been studied in which fibrosis, occurring without explainable reason, has resulted in complete absorption of the new growth.

With transplanted animal tumors which are undergoing absorption, the first change noted is death of the neoplastic cell, occurring first in scattered areas and finally involving the entire tumor; subsequently the necrotic material is in part liquefied, in part removed by scavenger cells, and the defect is finally healed by granulation tissue. What causes this cell death is a much disputed point, none of the several theoretical explanations which have been offered having as yet the support of a clear cut experimental proof.

The general body reactions noted in man during tumor recession are of several different types. The greatest number of spontaneous regressions have occurred following incomplete surgical removal of the tumor, next in order of frequency during some acute febrile process, and least frequently in connection

with some profound alteration in the metabolic processes of the organism such as extreme cachexia, artificial menopause, or the puerperium. ✕

When recession occurs after incomplete operation, search for a possible explanation becomes very confusing. ✕ The cases coming under this heading can best be explained by classifying them according to what has actually been done at the operation, and then considering the various possibilities under each subdivision.

✕ In the first subdivision of this broad group come the cases in which neoplasms have partly or completely receded after a simple exploratory laparotomy, the only tumor removed having been a small piece for histological diagnosis. In some of the histories of this group it is definitely stated that immediately after operation a high temperature developed, which continued without remission for several days. In this, as in all the subgroups of this division, we must consider the influence of the anesthetic, for it is possible that the removal of blood and tissue lipoids by the anesthetic might be an important element in the disappearance of the growth, though Gaylord and Simpson (126) as a result of their experimental work with mice came to the conclusion that removal of blood lipoids by an anesthetic stimulated growth. With the single exception that some of the cases have had high fever, no other fact leading to an explanation can be gathered from the data available. ✕

✕ In the second subdivision come the cases in which complete extirpation was impossible, though a large portion of the tumor was removed. But since pericancerous inflammation may extend for some considerable distance outside the true tumor, and since this tissue often cannot be distinguished at operation from the tumor, it is possible, and even probable, that in not a few of these cases the inflammatory tissue has been mistaken for tumor. The tumor having been removed, the pericancerous inflammation subsided and what had been erroneously regarded as neoplastic tissue thus disappeared. ✕ Another possibility is, that after incomplete removal the necessary manipulations and ligations so interfered with the blood supply of the remaining malignant tissue that death occurred from malnutrition. ✕

In the third subdivision, we have to deal with the question of heat externally applied. Practically all these cases are uterine carcinomata which, being surgically inoperable, were curetted and cauterized to ease the symptoms of discharge and hemorrhage. In some instances this operation was repeated several times. The inhibitory influence of heat (fever) upon the recurrence of neoplastic proliferation has been commented upon by many observers. Thus Krukenberg (4) has reported that patients with carcinoma of the uterus in whom fever appeared before operation showed recurrence in 48.3 per cent as compared with 58.5 per cent in those fever-free before operation. Lomer (85), in a most thorough and masterly survey, has drawn interesting deductions from the cases which he has compiled. It is true that the majority of the cases collected by him were uterine carcinomata, many of which were of the fundus and of a comparatively low grade of malignancy; still the period of freedom from recurrence after curettage and cautery, as recorded by this observer, is striking.

The occurrence of recession after acute infections has been commented upon in another paragraph. It has been shown experimentally (5) that comparatively low degrees of heat applied to animal tumors before inoculation, even for brief periods, distinctly retard the growth energy of these tumors, though others say that the growth energy is increased, and still others that heat has no effect. Bolognino (6), one of the many who has commented upon the relation of heat (fever) to spontaneous recession, has drawn attention to the fact that the amount of tumor still remaining before recession has occurred in the majority of cases has been small; however, as much as 375 sq. cm. of a cancer has disappeared. From the clinical standpoint, the important points in regard to heat are: (a) a temperature of from 104° to 105° F. (much lower than that of the actual cautery); (b) continuance of this for from forty-eight to ninety-six hours without remission.

The observation that a neoplasm may be absorbed subsequent to, or during, an acute infection, opens a most interesting field for speculation. The greater number of cases in this group have

occurred after an attack of erysipelas, an observation which has led to the use of toxins of the causative organism as a therapeutic measure. However, further observation shows that recession has also occurred after small-pox, pneumonia, malaria, and acute tuberculosis. No one organism is, therefore, specific in causing regression. In the histories of those cases that are given in detail, there is a common symptom, namely, high temperature, sustained without remission for several days. It is well to emphasize here that not every case of erysipelas, acute tuberculosis, or small-pox has a temperature as high as 104° F. continuously for from three to five days. Injections of bacterial toxins, while they do give rise to violent febrile reactions, are not characterized by the continuance of fever in full violence and without remission over a period of forty-eight hours.

Recession of growth during profound alterations in general metabolism is, in the present state of our knowledge, not susceptible of clear explanation. Those engaged in experimental cancer research have shown that animals in a poor state of nutrition are not good subjects for inoculation with tumor, the grafts either not growing at all, or proliferating very slowly and very often receding. This coincides with case reports reciting cessation or regression during cachexia. On the other hand, the recent study of Jaworski (7), made during the present world war, has shown that in man a deficiency of 50 per cent in the calories necessary to sustain equilibrium in weight, although it often results in the absorption of benign tumors such as myomata, may markedly increase the growth of malignant neoplasms. While poor health and cachexia are associated with loss of weight, emaciation due to starvation should not be construed as identical with cachexia or poor health. There is evidently some element other than the relatively simple one of food supply involved in the regressive change.

The influence of nutrition is more plainly shown in the several recorded instances of neoplasm arising during pregnancy and receding during the lying-in period. In two of the recorded cases, this sequence of events occurred in three successive pregnancies. It can be logically expected that an increase of nour-

ishment would cause rapid growth of a tumor, but the neoplasm having once started, why recession should occur upon a return to a more normal metabolic condition is, in the present state of our knowledge, unexplainable.

In another and smaller group, recession of a malignant tumor has followed the absorption of a transudate. These cases are best explained by cytotoxin or cytolyisin formation, even though such antibodies have as yet not been demonstrated by experimental means. The spontaneous regression of chorioepitheliomata is quite possibly due to such antibodies, as the tumor cells are not strictly autologous, half being derived from the male in distinction to ordinary tumors in which the cells are completely autologous.

It may be concluded that recession of a malignant growth can occur in either sex, at any age period, with any type of malignant tumor, and irrespective of the location of the growth. It occurs after a variety of conditions, a proof that no particular one is specific, and it is very probable that all the conditions noted are preliminary, so to speak, and act by depressing the proliferative energy of the malignant cell until the defensive forces of the body (cytotoxins, cytolyisins) are able to accomplish the final destruction. It appears that the most efficacious of all the many conditions which can bring about regressive change is heat, applied from without or occurring under the limited conditions of long duration and comparatively low degree.

The three examples which have come under the personal observation of the writer, and which are reported here for the first time, are given below.

The first, seen with Dr. J. A. Miller of New York City, is typical, there being many similar ones in the literature. It was a case of lymphosarcoma which underwent periods of recession, both with and without treatment, but subsequently terminated fatally.

Case 1. The patient was a Hebrew, forty-five years of age, whose occupation was that of clothing salesman. He was married and had three healthy children; his past and family history gave no facts ap-

plicable to his present condition; his habits, as regards alcohol and tobacco, were good, and he denied a venereal infection. On April 26, 1916, he first consulted Dr. Miller, complaining of pain in the left thigh and buttock. Upon physical examination, the anterior and posterior cervical chain of lymph nodes were found enlarged, and there was a mass the size of a walnut in the left pectoral muscle. The patient had noticed the lymphatic enlargement for five days, but had not paid much attention to it. On that day, his blood count, urine, and temperature were normal, and the Wassermann reaction negative. On April 28, the largest of the cervical lymph nodes, which was about 2 cm. in diameter, was excised, and a histological diagnosis of lymphosarcoma was made by the writer; this diagnosis was confirmed by Dr. F. C. Wood. The cervical lymph nodes continued to enlarge as did the mass in the pectoral muscle.

On May 5, 1916, he was admitted to the General Memorial Hospital. The diagnosis of lymphosarcoma was confirmed and enlarged retrosternal nodes were demonstrated in radiographs. Coley's fluid was given without result, and the patient was discharged May 24, 1916, unimproved. Between the date of his discharge and August, 1916, he had repeated x-ray treatments. Late in August he was seen by Dr. Harlow Brooks, who also confirmed the diagnosis of lymphosarcoma.

In September, 1916, he was admitted to Lebanon Hospital and treated with salvarsan in full dosage. The Wassermann reaction was negative on five different examinations, both with and without provocative treatment. At the time of his admission to Lebanon Hospital, all his nodes, cervical and inguinal, were much enlarged, and the mass in the pectoral muscle had increased greatly in size. After anti-syphilitic treatment, all the nodules except the mass in the pectoral muscle subsided; this mass was removed and a histological diagnosis of lymphosarcoma made. After leaving the hospital, he remained well for six weeks, during which time he received salvarsan every ten days, as well as intramuscular injections of mercury.

On January 2, 1917, his conditions was as follows: He was very much weakened and jaundiced. The nodes on both sides of the neck were enlarged to a diameter of about 5 cm., and his spleen was palpable at the umbilicus. At the site of removal (left pectoral muscle) a recurrence about 5 cm. in diameter was present, and a mass was found in the epigastric region also, approximately 10 cm. in diameter. Examination of the blood showed nothing but a moderately severe anemia.

His condition steadily grew worse, all of the enlargements increasing in size and new involvements of the inguinal nodes appearing. This condition persisted up to February 16, 1917, when suddenly all the tumors disappeared overnight (observations twenty-four hours apart) the spleen receding to the costal arch, and the mass in the abdomen becoming decidedly smaller. This recession persisted for one week, when an increase in size again occurred, the nodes having attained before his death on March 5, 1917, a size comparable with the conditions present before recession set in. No autopsy was obtained.

Repeated examinations of the blood made during the last month of life showed no evidence of leukemia in either the total white count or in the differential count of the leucocytes. With the exception of the last day of the disease, the temperature was normal throughout his illness.

The second case observed by the writer emphasizes one of the fallacies to be guarded against.

Case 2. The patient was a female, seventy-five years of age, who had suffered from five cerebral hemorrhages and was as a result bedridden. During the second year of her stay in bed, a nonulcerated mass developed at the outer canthus of the right eye, projecting about half a centimeter above the level of the skin. This gradually increased in size until at the end of two years' growth it had reached a diameter of 2.5 cm. A clinical diagnosis of epithelioma was made and the use of radium suggested, a V-shaped section being removed for diagnosis; at the time of removal, there was rather free hemorrhage which was stopped by ligature. The histological diagnosis was squamous-cell epithelioma. Seven days after the operation, the tumor commenced to recede and within another week disappeared. Five weeks later the patient died of pneumonia.

At autopsy, the site of the tumor was removed en bloc, and serial sections were made. Examination showed that a large blood-vessel entered the neoplasm at the line of incision for diagnosis, and that the disappearance of the growth had probably followed obliteration of a major portion of its blood supply.

Case 3. This case occurred in the practise of the late Dr. A. V. Deuring. The patient, a female aged fifty-seven, had been under my (G. L. R.) observation for eleven years. When she was thirty-seven years old (twenty years ago) she had backache, obstipation, loss of weight, and bearing down pains, and a physical examination carried

out at that time showed a large irregular mass apparently arising from the left side of the pelvic girdle. At an exploratory laparotomy, an inoperable condition was found; there was a mass estimated to be the size of an adult head arising from the lateral wall of the left pelvis, and to it the descending colon and sigmoid were firmly attached, while scattered over the peritoneum were innumerable secondary growths. One of these was removed from the omentum, but further than the extirpation of this piece no surgery was attempted. Microscopical examination proved the tissue to be a typical spindle-cell sarcoma.

Subsequent to the operation, a marked sciatic neuritis developed on the right side (pressure or metastasis?). For the sciatica, and without any idea of influencing the growth, the patient was given hot air baths (baking), the entire body being treated; thirty such treatments in all were given. To the surprise of all, instead of growing progressively worse the patient slowly improved until her health was completely restored two years after operation, the pelvic mass having also disappeared. In 1913, a blood Wassermann was negative, and in 1916, a luetin test was also negative. In December, 1916, the patient died of uremia. At autopsy, the anatomical diagnosis was pulmonary edema, mitral endocarditis, and chronic interstitial nephritis. There was no demonstrable lesion in the pelvis or in any of the abdominal viscera to indicate the former presence of a neoplasm.

Including those recorded in this paper, the writer has been able to collect a total of 302 cases in which recession, either temporary or permanent, has occurred in a malignant growth. These have been divided into three main groups: Group I consists of those reports which stand a most rigid scrutiny; group II contains those cases in which some slight question may be raised regarding the adequate control of all possible diagnostic errors, while the cases included in group III comprise those open to more or less grave doubt as to the correct diagnosis. Each of these main groups has been subdivided into (a) the group of complete recession, and (b) that of partial recession.

All the cases quoted below have been verified by a reading of the original article. There are still others in the Swedish, Russian, and Italian literature, but, the original articles not being available, these have not been quoted.

The criticism may justly be made that in many cases the period of observation has been too brief to permit the word cure to be used. Such criticism is beside the question; the fact remains that recession did occur, and the duration of such recession is not important from our present standpoint.

GROUP I

a. Complete recession of malignant tumor, carefully controlled

In the following paragraphs, the cases coming under Group I, subdivision (a), are briefly abstracted.

Mathews (8): A case of melanosarcoma originating in a pigmented mole of the back and involving the axillary nodes. The primary tumor and portions of the axillary nodes were removed, and the diagnosis confirmed by microscopical examination of both. Immediate return of the tumor was confidently expected, but, at the date of the report, the patient has been clinically well for two years. In a personal communication, Mathews states that the case was still free of recurrence three years and six months after operation.

Handley (9) has reported histological changes, which were recessive in character, in the metastatic deposits of a case of melanosarcoma. In his case, however, these changes did not progress to the degree of clinical manifestation.

Schuchard (10): An advanced carcinoma of the stomach, in which the organ was resected even though there were widespread peritoneal metastases with typical chyloform ascitic fluid. The diagnosis was confirmed histologically. Two and one-half years later this patient died; the autopsy showed no local recurrence, the peritoneal metastasis and ascitic fluid, also, having been absorbed. Pleurisy is given as the cause of death.

Godfrey (11): A case of carcinoma involving the tonsil and base of the tongue, so extensive as to be inoperable. The diagnosis was confirmed by microscopical examination. The patient was sent home to die, the only treatment given being antiseptic mouth washes. When seen, eighteen months later, there was no trace of the original tumor.

McKay (12): A scirrhus carcinoma of the breast, diagnosed microscopically, was removed in 1904. In January, 1906, there was a local recurrence which was treated with x-ray. The disease gradually

progressed until in August there was a pleural effusion and involvement of the opposite breast, the patient at that time being in a desperate condition. She continued to be practically moribund until December 28, 1906, when a sudden and overnight improvement occurred with the rapid absorption of the entire tumor area. Three weeks later the patient died (92).

Noble (13): A case histologically diagnosed as chorioepithelioma. The tumor had perforated the fundus uteri and was attached to the bladder. During the course of a hysterectomy, it became necessary to leave a portion of the tumor attached to the bladder. Examination sixteen months after operation showed that the patient was clinically well, and that no mass was palpable. Convalescence was febrile for six days.

Watson (14): A tumor directly beneath the chin was removed and examined histologically, the diagnosis being round-cell sarcoma. Later the tumor recurred in both right and left cervical chains of lymph nodes, and those on one side were removed. During convalescence from this operation, a violent attack of erysipelas occurred. Subsequent to the attack of erysipelas, the nodes on the other side spontaneously receded.

Gould (15): A carcinoma of the left breast was removed, and the diagnosis was confirmed histologically. A salpingo-oophorectomy was performed five months after the breast was amputated, and shortly thereafter appeared a recurrence in the breast scar and in the supra-clavicular nodes. Two and a half years later there were other recurrences and metastases, the liver also being much enlarged; at that time the patient was feeble and emaciated. For the next few years no treatment was given, yet the liver became smaller and all the recurrences disappeared except a small one in the breast scar. Subsequently the patient entirely recovered.

Weindler (34): Three cases, all of which were diagnosed microscopically. One was a glandular carcinoma of the fundus of the uterus, the other two were cervical uterine carcinomata. In all the cases, the treatment was curettage and cauterization. The patients, all of whom were inoperable cases when first seen, were clinically well from six to seven years after the palliative operation.

Ladinski (125) has collected a series of cases of undoubted carcinoma of the uterus (verified microscopically) which were apparently cured by a simple curettage. In eighteen of these, the curettage was followed by hysterectomy; in four no hysterectomy was performed.

Six of the cases were free of recurrence from six months to four years; in five the period of cure was not given.

Boldt (35): A case of inoperable abdominal carcinoma (the diagnosis being verified by histological examination of the involved omentum) was autopsied "some years later" and no carcinoma found.

Marchand (39): A case of chorioepithelioma, which had perforated the uterine wall and involved the pelvic tissues, the uterine vein being thrombosed by tumor emboli. The diagnosis was confirmed by histological examination. Incomplete removal of the tumor was the only surgical possibility and this was done. One year later, the patient died, and at the autopsy no recurrence was demonstrable.

Riesel (116): A case of chorioepithelioma with an atypical microscopic picture. There was invasion of the superficial uterine muscle. The patient remained well one year after a very thorough curettage.

Bolognino (40): A case of round-cell sarcoma of the axilla, with histological confirmation of the diagnosis. The tumor recurred after removal, the recurrence being removed incompletely by curette. Ten days after the curettement a very severe attack of erysipelas supervened and the tumor remnants disappeared. Thirteen months later, there was no return of the neoplasm.

Ibotson (41): In 1914, a female aged forty-five years had her breast amputated for carcinoma. In 1916, there was a recurrence in the scar and metastasis had occurred in the uterus; no further surgical interference was deemed advisable. In February, 1917, the nodules in the scar had disappeared, and the uterus had returned to a clinically normal condition. The author has also reported other cases in which recession followed oophorectomy.

Prochownik (59): Two cases of uterine carcinoma, both diagnosed microscopically, and considered inoperable. In order to relieve the symptoms of hemorrhage and discharge, both of the cases were curetted and cauterized. Upon examination, four and six years after operation, both cases were clinically free from all evidences of carcinoma.

Littauer (60): Six weeks after an abortion, there occurred irregular periods of bleeding from the uterus. Physical examination showed a mass "the size of a cherry" in the posterior wall of the uterus; this mass was removed and a histological diagnosis of chorioepithelioma was made. No hysterectomy was performed. Ten weeks later a second curettage showed no histological abnormalities. One year later, the patient was clinically well.

Grofe (61): A case of chorioepithelioma localized in a small area of the uterus, the tumor being palpable and a small piece having been

removed for histological diagnosis. Eight days later, the entire uterus was removed, and though it was cut in serial section no tumor could be demonstrated.

Langhans (62): A nodule which developed in the vagina after *accouchement* was removed for microscopic examination and found to be a chorioepithelioma. The nodule recurred in fourteen days, but shortly thereafter it suppurated and healed. Ten months later the patient was well.

Horman (63): A woman seven and a half months pregnant aborted. She was curetted, microscopical examination of the curettings showing chorioepithelioma. Shortly afterwards metastasis appeared and she was sent home to die. One year later, both the tumor and metastasis had disappeared. Subsequently she had a normal pregnancy.

Guinard (113): A case of carcinoma of the breast complicated by a uterine fibroid. Castration was performed during a hysterectomy. The breast tumor completely receded.

Three cases of malignant growths, in which castration was followed by recession, are quoted by Beatson also (118).

Johr (115): A sarcoma of the axilla with many metastases; the diagnosis was confirmed microscopically. The woman was pregnant and an abortion was induced, whereupon all the masses disappeared. Death occurred six weeks after absorption of the growths.

Lilienthal (119): An intercostal recurrence of a mammary carcinoma verified microscopically, which receded completely. This case, to the personal knowledge of the present writer, is still free of recurrence seven years later.

Powers (123): A fibrosarcoma of the skull, histologically diagnosed, which receded, together with its secondary deposits, after partial removal.

Kutzner (67): An eighteen-year old girl with a round-cell sarcoma of the breast had many metastatic deposits in the subcutaneous tissues of the body. The diagnosis was confirmed by histological examination. She developed an acute infection of the lung (pneumonia?) and all of the neoplastic deposits disappeared.

Kleeblatt (68): A case of primary lymphosarcoma, behind the auricle of the ear, and inoperable when seen. The diagnosis was confirmed by microscopical examination. The patient was purposely inoculated with erysipelas and complete recession of the tumor followed.

Fleischmann (69): Two and a quarter years after the delivery of a hydatiform mole, nodules were found in the vaginal wall and uterus.

The vaginal nodules were removed and examined histologically, the diagnosis being chorioepithelioma. Ten months later, there was no evidence of recurrence.

*Lick (70): An inoperable uterine carcinoma, microscopically diagnosed. The case was curetted and cauterized. Four years after operation, there was no return of symptoms and examination of the uterus showed a normal condition.

Jordan (82): An epithelioma of the cervix, which was histologically diagnosed, was treated with cautery and curettage. The entire uterus was removed two and a half years later, and when examined microscopically, no tumor could be found.

von Franque (83): A case of chorioepithelioma was curetted. The diagnosis was made by microscopical examination. For several days the patient had a temperature of 41°C. Twenty-nine days later, she was again curetted and no chorioepithelioma was found in the curettings. Subsequently she bore two healthy children.

Another case of chorioepithelioma verified by two curettages with histological examination. Twenty days after the last curettage, a hysterectomy was performed. The uterus was cut in serial sections and no chorioepithelioma found.

Gould (84): A carcinoma of the superior maxilla, verified by histological examination, recurred three months after operation. The recurrence was inoperable. Three months later, without any treatment, the recurrence had been completely absorbed.

Lomer (85): A case of carcinoma of the uterus, in which, during removal of the uterus, the broad ligaments were found infiltrated with tumor. In spite of incomplete removal the patient was well seven years later and there was no evidence of malignant growth.

Lomer has collected a series of 213 cases of recession of malignant growths, occurring chiefly after the application of the cautery; his article is intensely interesting and well worth study. Of the cases quoted by him, 10 have been considered separately in this abstract, and 103 have been excluded from consideration because of lack of detail or evident error in diagnosis; the remaining 100 have been accepted as *bona fide*. Of these 100 cases, 40 fall under the present group I (a); of the remainder, 20 come under group I (b); 17 under group II (a); 15 under group II (b); 8 under group III (c). They have not been quoted individually.

Clarke (86): An inoperable carcinoma of the stomach with metastasis in the mesenteric nodes verified by histological examination. The patient was perfectly well nine years after an exploratory laparotomy.

Chrobak (87): A case of carcinoma of the cervix uteri with a histological diagnosis. Eleven years after curettage and cautery, the patient was well and without evidence of tumor.

A case of carcinoma of the uterus, verified microscopically, which was curetted and cauterized in 1884; the patient was still well and tumor free in 1905.

Proescher (96): A carcinoma of the gall bladder, microscopically verified. The gall bladder was drained. The fistula persisting the patient was again operated several months later and the gall bladder removed. Histological examination of the gall bladder showed replacement of the entire carcinoma with hyaline masses; no tumor cells were demonstrable.

Martin (108): A female, forty years of age, with an epithelioma of the cervix verified by microscopical examination. Operation was refused. Twenty-two years later, the patient was alive and well without evidence of tumor.

Bretschneider (24): An inoperable carcinoma of the uterus, the diagnosis being confirmed microscopically, was curetted and cauterized. One year later the patient had so improved that a radical operation was unsuccessfully attempted. Curettage and cautery were again employed. The curetting from the second operation also showed glandular carcinoma. One year after the second operation the patient was clinically well.

b. Partial recession of malignant tumor, carefully controlled

Brief abstracts of the cases falling in this group are given below:

Rotter (32): A female of thirty-one, with the clinical diagnosis of polyposis of the rectum complicated by an adenoma malignum, the diagnosis being confirmed histologically, was operated upon and the tumor removed. Recurrence took place in three months, the histological diagnosis of the recurrence being the same as for the primary tumor. The first recurrence was removed only to be followed by a second one which was not removed. Six months after the last opera-

tion, the patient was clinically cured. Death occurred three years after the last operation. At the autopsy, it was found that the rectal tumor had completely disappeared, the only remaining evidence of malignancy being a small metastasis in the iliac bone. Histologically this metastasis was of the same type as the primary tumor.

Bolognesi (16): A case of multiple basal-cell epitheliomata of the face and lip, in a woman. The diagnosis was made histologically. One of the multiple growths completely receded. A biopsy of the healed lesion showed an extensive and marked fibrosis.

Dahmen (18): A case where at autopsy a primary carcinoma of the stomach was almost completely healed, though there were enormous metastases in the liver.

Schwarz (5): An epithelioma of the head arising from a sebaceous cyst in which healing was taking place. The recession, however, was only demonstrable histologically.

Martin (120): An epithelioma of the eyelid, almost completely healed as a result of calcareous degeneration.

Gravers and Price (52): A case of hemangioendothelial blastoma occurring in the mouth of a young negress. The tumor developed during her first pregnancy, and almost completely receded during the lying-in period. The tumor was finally removed, but recurred.

Hodenpyle (57): A case of recurrent carcinoma of the breast with metastases in the liver which receded after the appearance of a chyliform ascitic fluid. The patient subsequently died of carcinoma.

Butlin (58): A case of metastatic epithelioma in the lymph nodes of a chimney sweep. A thorough examination failed to show any malignant or other growth on the scrotum or elsewhere in the body. The primary tumor had evidently completely receded.

Watson (19): A case of pedunculated neoplasm of the back in the region of the scapula occurring in a female thirty-six years of age. The tumor was removed but not examined histologically. Four days after operation, the growth was as large as before operation, though not pedunculated. Portions of the recurrence were removed and examined histologically, the diagnosis being round-cell sarcoma. Seven days later an unsuccessful attempt was made to remove the recurrence. The convalescence after the second operation was febrile. One year later, the tumor was much smaller, approximately the size of the original growth and again pedunculated.

Wells (20): A case of carcinoma of the uterus, verified by microscopic examination, and found by exploratory laparotomy to be inoper-

able. Four and a half years after operation, the tumor had infiltrated the vagina. One year later the patient died and at the autopsy the peritoneum was found studded with small whitish areas, while the primary tumor had disappeared. Histological examination of the whitish areas covering the peritoneum showed carcinoma with extreme calcareous infiltration.

Jacobsthal (17): A case of epithelioma in a male forty-five years of age. Because of the peculiar clinical appearance of the growth the tumor was cut serially, and it was found that the malignant tissue itself consisted of a ring shaped area, the central cavity of the ring being filled with a dense connective tissue. This histological picture is interpreted as showing that the central portion of the growth had healed, though the periphery had kept on growing.

Handley (48): Four cases in which spontaneous fracture of bones occurred following metastatic tumor formation. Although all the cases subsequently died of the malignant condition, the fractures healed and the metastatic tumors which gave rise to the fractures disappeared.

Longcope (25): A male in whom tumors of varying size were found at operation scattered throughout the peritoneal cavity, chiefly in the gut and mesentery; the condition was considered inoperable, and the abdomen closed. Directly after the operation, the patient improved for a period, then died. At autopsy, it was found that most of the nodules had almost disappeared, though histological examination showed diffuse lymphosarcoma in practically all the abdominal viscera.

Osler (22): A carcinoma of the breast was removed in 1897. One year later, secondary deposits appeared in the right eye, and there were also metastases in the sternum, and a pleurisy with effusion. Paraplegia due to spinal cord metastasis developed, the patient becoming very much emaciated and being confined to bed for months. In 1900, recession had so far advanced that the paraplegia had disappeared and the ocular symptoms had so improved that sight was to some degree restored. The sternal metastases, furthermore, had been absorbed. The patient was able to go about at this time, but eventually died of carcinoma.

In a second case, a breast was removed for carcinoma in 1898. In 1899, a spinal cord metastasis developed, causing a complete paraplegia. There was also a recurrence in the scar and in the lymph nodes of the neck. Both the paraplegia and the other secondary in-

voluments temporarily entirely disappeared; eventually, however, the patient died of carcinoma.

Could (21): A scirrhus carcinoma of the breast, diagnosed microscopically, recurred two years after operation. The recurrence was removed, but another developed two years later. The second recurrence was removed, the menopause occurring at the same time. A third recurrence developed and with it metastasis in the chest and femur. The patient then apparently became moribund, and the various neoplastic deposits disappeared. Recession was temporary, however, death occurring several years later of carcinoma.

(Footnote in original article (23)): A woman of thirty-six years of age, who had had several children, was again pregnant seven months, when masses developed in the breast, axillary nodes, introitus of the vagina, uterine cervix, and in the pouch of Douglas. An abortion was induced with glycerin, and ten days afterward all the masses, except that in the cervix, had disappeared. This mass was removed and examined histologically, the diagnosis being sarcoma of type not specified. Six weeks later the patient died suddenly in collapse. There was no autopsy.

Schkarin (71): A case of round-cell sarcoma in a six year old girl. At the time of operation, the peritoneum was found greatly thickened and there were innumerable metastases scattered over the peritoneum and mesentery. Part of the peritoneum and one of the metastases were excised and examined histologically, the microscopical diagnosis of round-cell sarcoma being made. The condition was so far advanced that the primary tumor could not be definitely located at the operation. For four days after operation, the patient had a violent fever and then died. At autopsy, a round-cell sarcoma of the jejunum 2.5 cm. in diameter was all that was left of the extensive involvement found at operation. The temperature was not due to sepsis.

A five year old child with a tumor in the sternum and in the abdomen. Five days after an exploratory laparotomy both masses disappeared, only to recur and cause death four weeks after operation. The histological diagnosis was sarcoma.

Walsehe (72): A carcinoma of the breast of two years' duration. A cerebral metastasis producing the symptoms of apoplexy occurred, which caused death ten weeks later. At autopsy, the primary tumor was found completely absorbed, though the metastasis in the brain was evidently growing.

Kaposi (117): A tumor of the upper jaw with multiple skin metastases. A piece was excised for histological examination, the microscopical diagnosis being lymphosarcoma. All the skin metastases quickly disappeared after the excision of the piece for diagnosis. Death subsequently occurred from lymphosarcoma.

Peterson and Colmers (105): A carcinoma of the pylorus, which was resected, several carcinomatous nodes being left behind. The diagnosis was confirmed by histological examination. One and a half years later, the patient died of intestinal obstruction due to an umbilical hernia. At autopsy, the cancerous lymph nodes had disappeared though there were small metastases in other organs.

Saar (104): While studying the regressive changes of the breast associated with senility, this author discovered a minute carcinoma undergoing recessive alteration.

Randolph (101): An endothelioma of the arm of three years' duration, growing very rapidly in the last six months, was removed. Histologically, the tumor showed a subsiding acute inflammation which was leading to fibrosis and healing.

Friedman (93): A tumor of the lip which receded completely, only to be followed by involvement of the lymph nodes. Removal of the affected nodes showed metastatic carcinoma, the primary tumor having healed completely. Death occurred from carcinomatosis.

Wells (92): A carcinoma of the breast exhibiting recessional changes in the metastases after the primary tumor had been removed.

Cleland (111): An epithelioma of the neck in which distinct recession and healing were demonstrable histologically.

Merkins (quoted by Hendry (110)): A carcinoma of the lip which had completely healed, though there were metastases in the regional lymph nodes.

Eissemenge (114): A case in which tracheotomy was done to relieve the dyspnea produced by a round-cell sarcoma of the tonsil. The tumor completely disappeared. Metastases in other regions finally caused death.

Handley (121): A carcinoma of the breast of twenty-eight years' duration, in which secondary deposits repeatedly disappeared.

Gould (122): Almost complete recession of a carcinoma of the breast after oöphorectomy.

GROUP II

a. Complete recession of malignant tumor, open to slight question

The cases coming under this group are abstracted below:

Bolognino (26): A case of adenocarcinoma of the testis which previous to operation had been treated ineffectually with potassium iodide. After operation, a metastasis developed in the opposite testis. This metastasis became ulcerated and an attack of erysipelas followed, after which the tumor disappeared, only to recur later. This time the recurrence was partly removed, and ten days later, a second and violent attack of erysipelas occurred. Ten months afterward the remaining tumor had entirely disappeared.

Czerny (27): In resecting a sigmoid for carcinoma, the line of excision passed through cancerous tissue. In spite of the incomplete operation, the patient was free of symptoms five years later.

In resecting a carcinoma of the colon in which the tumor was adherent to the small gut, part of the tumor was left behind. Four years later the patient was clinically well. These tumors, of course, might have been due to a diverticulitis of the gut, the inflammatory and hyperplastic lesions of which often are called malignant.

Theilhaber and Edelberg (28): Three cases of carcinoma of the uterine cervix, in which it was not possible totally to remove the tumor, portions of involved broad ligament being left behind. These cases were clinically well seven, five, and four years respectively, after operation.

Sigg (66): A tumor of the breast, together with enlarged axillary nodes, was extirpated; the growth is said to have been "malignant." One year later there was a recurrence, which was removed, and a second recurrence took place six months afterward. Shortly thereafter, the patient developed pulmonary tuberculosis, and, as this disease advanced, the neoplastic tissue was absorbed completely. Death occurred from tuberculosis. There was no autopsy.

De Gaetano (74): A patient with an epithelioma over the left frontal eminence was attacked by erysipelas two years after the onset of the tumor; the growth disappeared, to return three years later, following trauma at its former site. This recurrence was twice removed, reappearing, however, each time. After the last recurrence there occurred a violent attack of erysipelas, and this was followed by final disappearance of the tumor.

Koster (88): A sixteen year old boy, with a tumor of the orbital fossa, to whom iodide of potash had been given without result. The histological diagnosis was sarcoma. Only a portion of the tumor could be removed. Five years later, the tumor had completely receded and the patient was entirely well.

Warring (89): An inoperable carcinoma of the gall bladder, verified microscopically, which receded, the patient being alive and free of all symptoms two years after an exploratory laparotomy.

An inoperable carcinoma of the caecum, verified microscopically, the patient being alive and well seven years after an exploratory laparotomy.

Loeffler (90): A case of carcinoma of the breast, verified microscopically, which receded when temperature (fever) occurred during the course of a malaria.

Riffel (91): An epithelioma of the face which receded after an attack of small-pox.

Schlimpert (53): A case of tumor of the liver which was entirely removed, the histological diagnosis being epithelioma. At the time of operation, radiographs showed shadows in the lungs which were taken to be metastatic deposits. Three years later, the lung shadows had disappeared and there was no recurrence of the liver tumor. Whether this tumor was primary in the lung or in the liver is immaterial from the standpoint of the present review.

Zagger (29): A male, with a papilloma of the gum, noticed that the tumor was suddenly commencing to grow rapidly. When first seen by the physician, the tumor was as large as an egg and inoperable. A portion of the growth was removed for histological diagnosis, the report being "malignant." The patient became extremely cachectic and finally almost moribund, in which condition he remained for six weeks. The tumor completely receded during this period, and the patient then recovered his usual good health.

Brettauer (30): A carcinoma of the uterus, in which the uterine arteries had been ligated when the intended operation (hysterectomy) had to be abandoned because of the collapse of the patient. Since then, time not specified, the patient has been well.

A carcinoma of the cervix curetted and cauterized repeatedly, the patient being reported as clinically well.

A carcinoma of the ovary found to be inoperable by exploratory laparotomy. The patient was alive and well two years later.

Webb (73): A carcinoma of the lip was twice operated on, and again

recurred; the third recurrence was inoperable. The final metastasis receded and had not recurred eight years later, when the patient died.

von Hanseemann (107): A malignant adenoma of the rectum (diagnosis confirmed histologically) which at first grew rapidly, but then receded completely, disappearing eight years after the onset.

Hall (103): A hypernephroma within the capsule of the kidney, the tumor being entirely necrotic.

Fleischmann (102): A carcinoma of the cervix, microscopically confirmed, in which part of the tumor only was removed. There was profuse postoperative bleeding followed by temperature. Eleven years later the patient was free of recurrence.

A carcinoma of the cervix, microscopically confirmed, with incomplete operation; the patient was free from recurrence eleven years after operation.

Perrin (97): A carcinoma of the breast which receded after an operation for goiter. The operation wound had become infected and extensive suppuration with high temperature had followed.

Richardson (95): A male of thirty, with a carcinoma of the bladder, verified microscopically, was operated and the tumor removed. Shortly thereafter the tumor recurred, but the patient refused further operation. Two years later, the tumor had disappeared, and four years later there was no recurrence.

Wells (92): A male of sixty-three, with a colloid carcinoma of the caecum in the region of the appendix. It was declared inoperable and nothing was done at the exploratory laparotomy. One year later, he was again operated on for fecal fistula and it was found that the cancer had become calcified. Thirteen years after the first operation death occurred; the autopsy disclosed an extensive colloid carcinoma involving all the abdominal viscera.

b. Partial recession of malignant tumor, open to slight question

The succeeding paragraphs give in abstracted form the cases classed under this heading:

(Footnote in original article (36)): A female, during her first pregnancy, noticed a tumor in the breast which grew rapidly during the pregnancy, but which subsided almost to the point of complete disappearance during the lying-in period. She again became pregnant and the neoplasm grew as before, but did not recede to the same degree during the lying-in period. A third pregnancy occurring, the

tumor again grew rapidly but this time did not recede at all. Death supervened, and the histological diagnosis at autopsy was sarcoma, type not specified. It may be asserted that it was only during the last pregnancy that the tumor really became malignant, an objection which cannot be answered.

Williams (37): A case with enlarged lymph nodes in the neck, histological examination showing metastatic epithelioma, though there was no evidence of tumor in any region of the body. The inference is, that the primary tumor had completely healed.

Quossow (38): An eleven year old boy had a mass on the right side of his throat so large as to cause dyspnea. A tracheotomy was performed and the tumor disappeared in nine days, only to recur subsequently with metastasis. The histological diagnosis at autopsy was a polymorphous-cell sarcoma.

Williams (106): Seven cases of carcinoma of the breast in which the primary tumor receded while the secondary deposits progressed and killed the patient. Such recession may be due to a central necrosis from vascular thrombosis with absorption of the necrotic tissue.

Graham (94): A carcinoma of the breast, microscopically verified. The primary tumor was removed, and at death six years later healing by fibrosis was taking place in all the metastases.

GROUP III

a. Complete disappearance of malignant tumor, poorly controlled

The cases coming under this classification are abstracted below:

Mundi (50): A case of carcinoma of the vagina in a young girl which spontaneously receded. No further details are given.

Sanger (54): A case of epithelioma of the cheek due to a sharp tooth. The tooth was extracted and one-half the tumor removed for examination. The microscopical diagnosis was epithelioma. The portion of the tumor which was not removed disappeared in four weeks. Gaylord and Clowes, in their summary, consider this case as "bona fide;" however, syphilis has not been excluded. The histological picture resembled more an atypical epithelial proliferation, such as is often seen in wound healing and is more exactly duplicated by Scharlach R (55), than it does an epithelioma.

Crosbie (56): A tumor said to be malignant, in a male seventeen years of age. The neoplasm presented itself as an ulcer the size of a "sixpenny piece," having raised edges and numerous warty nodules; a portion was excised and reported as "malignant." The patient at the same time had an eczema (?). Eight months later, both tumor and eczema disappeared. Gaylord and Clowes consider this also as a "bona fide" case, but the histological description and the strong suspicion of syphilis have not been properly controlled.

Klotz (64): Six cases of carcinoma of the uterus with palliative operation, curettage, and cautery. The tumors are said to have disappeared.

Frankel (65): Three cases of uterine carcinoma treated with cautery and curettage. The tumors are described as having disappeared.

Both of these reports (Klotz and Frankel) are given later notice by Lomer (85).

Haidenhain (47): A case of ovarian tumor with peritoneal metastasis; there is no histological diagnosis or gross description. The patient is said to be well and living twenty years after an exploratory laparotomy.

A patient with a malignant abdominal neoplasm (no details given) said to be well six years after an exploratory laparotomy.

Coley (44): A carcinoma of the breast covering 150 sq. cm. which disappeared six weeks after a very severe attack of erysipelas. Nine years later there had been no recurrence. No histological examination was made in this or in any of the following cases described by Coley.

A case of sarcoma of the neck in which recession of the tumor occurred after an intentional inoculation with erysipelas. This case was observed for eight years and showed no recurrence.

A case of epithelioma of the face and cheek, in which the tumor disappeared following a violent attack of erysipelas.

A case of epithelioma of the face clinically cured two months after an attack of erysipelas, and still clinically well six months afterwards.

An epithelioma of the nose of eight years' duration, in which the tumor was absorbed after a violent attack of erysipelas. When the patient was seen several years later there was no recurrence.

Czerny (42): A carcinoma of the breast recurrent one year after operation. The recurrence was removed, a severe attack of erysipelas occurring directly afterward. This patient was observed for twenty years and there was no recurrence.

Theilhaber (43): A case of carcinoma of the breast with involvement of the pectoral muscle, the diagnosis being confirmed histologically. Shortly after the operation the axillary lymph nodes became involved, though they were not examined histologically. The involvement of the nodes disappeared after hot air treatment.

Vidal (49): Three cases of malignant growth in which recession occurred after a temperature of 40°C. He gives but the outline of one case and no details of any of the others. The author asserts that spontaneous recession was brought about in three dogs bearing sarcomata, by destroying the heat regulating center.

Czerny (42): A case of carcinoma of the uterus which was treated palliatively with curette and cautery. Five years after operation there was no clinical evidence of carcinoma.

Theilhaber and Edelberg (45): A case of carcinoma of the ovary, which could not be completely removed. At the operation, an exudate was found in the pelvis. In spite of incomplete removal the case went on to complete recovery.

Czerny (46): A sarcoma of the nose appearing after an injury and recurring after removal. The recurrence was simply curetted, and the patient has been clinically well for ten years. This tumor was probably a granuloma.

Billroth (75): A carcinoma of the breast as large as an apple which spontaneously receded. The patient was free of all symptoms one year later.

Mohr (76): A tumor of the cheek which followed irritation by a ragged tooth. The growth was first noticed in 1896. It steadily increased in size and four years later gave rise to difficulty in opening the jaw. The condition was inoperable, and nothing was done save to extract the tooth. Two and a half years later the tumor had entirely disappeared. There was no histological examination.

Roux (77): A carcinoma of the stomach with many metastases scattered over the peritoneum. The primary tumor was resected; three years later the patient was alive and well.

Widmer (78): A tumor of the hand, which was clinically a carcinoma, though no microscopical examination was made; it receded spontaneously upon exposure to sunlight.

Coley (100): A round-cell sarcoma of the neck with five recurrences. An attack of erysipelas set in after the last recurrence, followed by disappearance of the tumor. No return of the tumor seven years later.

Recurrent round-cell sarcoma of the tonsil, involvement of the lymph nodes. After an attack of erysipelas the entire lesion receded.

Gerster (109): A sarcoma of the lower end of the femur. The leg was amputated and recurrence took place in the stump. An attack of erysipelas was followed by spontaneous regression of the recurrence.

Warthin and Spitzley (112): Three cases which appeared clinically to be malignant, and which receded after incomplete operation. The authors frankly doubt the validity of the cases.

Could (122): An inoperable carcinoma of the cervix, so extensive as to result in paralysis of the lower limbs, completely receded.

A recurrent carcinoma of the breast which receded after oöphorectomy. The patient was still free ten years after operation.

Fischel (124): A perithelial sarcoma, microscopically verified, which liquefied. The fluid was evacuated by incision, the tumor remnants not being removed. Subsequently the man was clinically well.

b. Partial disappearance of malignant tumor, poorly controlled

The succeeding paragraphs give the abstracts of the cases in this group.

Hutchinson (79): A hard tumor of the breast, of thirteen years' duration, had completely receded at the time of the patient's death, though the autopsy showed innumerable visceral metastases.

Paget (80): A female of fifty-nine years had a postoperative recurrence of a mammary carcinoma. The recurrence temporarily receded when tuberculosis developed.

Billroth (81): A carcinoma of the breast of seven years' duration, which for the last two years had been gradually receding in size without ulceration, though involvement of the lymph nodes had been progressing.

Steudel (98): Subsidence of a carcinoma of the stomach after gastroenterostomy.

Lindner (99): Subsidence of a carcinoma of the stomach after gastroenterostomy.

CONCLUSIONS

TABLE 1

TABLE I			
TYPE OF TUMOR	ORGAN INCIDENCE	ASSIGNED CAUSE OF ABSORPTION	
Carcinoma and epithelioma.....	Breast.....	28	
	Bone.....	4	Acute general infection. 17
	Gastro intestinal tract, mouth excepted.....	13	Fibrosis or calcification. 6
			Heat..... 54
			Incomplete operation... 58
	Liver and gall bladder. 3		Nutritional..... 12
	Mouth, tonsil, lip, and cheek.....	9	Not given..... 28
	Not mentioned.....	6	
	Skin.....	11	
	Testis.....	1	
	Urinary bladder.....	1	
	Uterus and appendages. 82		
Chorioepithelioma....	Uterus and appendages. 7		Incomplete operation... 6
			Acute infection..... 1
Endothelioma.....	Arm.....	1	Nutritional..... 1
	Mouth.....	1	Fibrosis..... 1
Hypernephroma.....	Kidney.....	1	Not given
Sarcoma.....	Abdomen.....	3	Incomplete operation... 5
	Axilla.....	1	Not given..... 7
	Back.....	1	Nutritional..... 2
	Bone.....	4	Heat..... 10
	Breast.....	2	Acute general infection.. 9
	Ear.....	1	
	Eye.....	1	
	Neck.....	3	
	Nose.....	1	
	Not mentioned.....	4	
	Skin.....	1	
	Tonsil.....	2	

Lomer's cases (100 in number) are not included in this table because they have not been given in detail in this summary. The groups in which heat is assigned as the cause of recession include cases from other groups in which fever or externally applied heat are recorded.

The statistical data to be gathered from the cases recorded in the literature are presented in the table. If all the cases are considered collectively, without regard to the probable accuracy

of the various reports, it will be noted that malignant epithelial tumors are present in the largest number, with malignant connective tissue tumors second in order of frequency. The causes of recession as given by the various authors, or as determined by the history of the case, show an almost equal number following incomplete operation and heat. Whether this heat be the result of some general acute infection such as erysipelas, tuberculosis, or pneumonia, or whether it be applied from external sources, has not been considered from the statistical standpoint.

The chief lesson to be learned from the summary of cases here presented is that occasionally, perhaps with greater frequency than is ordinarily imagined, a malignant tumor will spontaneously recede. That such a regression does ever occur suggests that there may be found some method of bringing it about at will, even though this cannot be accomplished at the present time.

The occurrence of partial or complete spontaneous recession should make one very critical in judging new therapeutic procedures, that they may not be falsely credited with the results produced by forces of the nature of which we are for the present entirely ignorant.

REFERENCES

- (1) WOOD: Jour. Am. Med. Assn., 1916, lxvi, 94.
- (2) WOLFF: Lehre von der Krebskrankheit, Jena, 1911, ii, 468.
- (3) HANOVER: Das Epitheliom, usw., 1852.
- (4) KRUCKENBERG: Ztschr. f. Geburtsh. u. Gynäk., 1889, xxiii, 103.
- (5) TSURUMI: Jour. Path. and Bacteriol., 1915, xx, 76.
- (6) BOLOGNINO: Ztschr. f. Krebsforsch., 1908, vi, 261.
- (7) JAWORSKI: Wien. klin. Wchnschr., 1916, xxix, 1646.
- (8) MATHEWS: Ann. Surg., 1915, lxii, 115.
- (9) HANDLEY: Arch. Middlesex Hosp., 1906, vii, 68.
- (10) SCHUCHARDT: Deutsch. Gynäk. Kongress, 1901, xxx, 664.
- (11) GODFREY: Brit. Med. Jour., 1910, ii, 2027.
- (12) MCKAY: Brit. Med. Jour., 1907, ii, 138.
- (13) NOBLE: Amer. Jour. Obst., 1902, xlv, 290.
- (14) WATSON: Lancet, London, 1902, i, 300.
- (15) GOULD: Clin. Soc. Tr., London, 1897, xxx, 205.
- (16) BOLOGNESI: Arch. f. klin. Chir., 1910, xciv, 705.
- (17) JACOBSTHAL: Arch. f. klin. Chir., 1907, lxxxiv, 325.
- (18) DAHMEN: Ztschr. f. Krebsforsch., 1905, iii, 298.
- (19) WATSON: Lancet, London, 1902, i, 300.
- (20) WELLS: Amer. Jour. Obst., 1908, lvii, 403.

- (21) GOULD: Clin. Soc. Tr., London, 1897, xxx, 205.
- (22) OSLER: Brit. Med. Jour., 1906, i, 3.
- (23) Footnote in original article: Berl. klin. Wehnschr., 1894, xxxi, 726.
- (24) BRETSCHNEIDER: Arch. f. Gynäk., 1910, xcii, 107.
- (25) LONGCOPE: Bull. Ayer Clin. Lab., Penn. Hosp., 1910, No. 6, 1.
- (26) BOLOGNINO: Ztschr. f. Krebsforsch., 1908, vi, 261.
- (27) CZERNY: Ztschr. f. Krebsforsch., 1907, v, 27.
- (28) THEILHABER AND EDELBERG: Ztschr. f. Krebsforsch., 1913, xiii, 473.
- (29) ZANGGER: Cor.-Bl. f. schweiz. Aertze, 1904, xxxiv, 626.
- (30) BRETtauER: Amer. Jour. Obst., 1908, lviii, 403-406.
- (31) THEILHABER AND EDELBERG: Ztschr. f. Krebsforsch., 1913, xiii, 478.
- (32) ROTTER: Arch. f. klin. Chir., 1899, lviii, 357; 1900, lxi, 881.
- (33) FRANKEL: Monatschr. f. Geburtsh. u. Gynäk., 1910, xxxii, 180.
- (34) WEINDLER: Zentralbl. f. Gynäk., 1907, xxxi, 632.
- (35) BOLDT: Amer. Jour. Obst., 1908, lvii, 403.
- (36) Footnote in original article: Berl. klin. Wehnschr., 1894, xxxi, 726.
- (37) WILLIAMS: Natural History of Cancer, New York, 1908, p. 426.
- (38) QUOSSOW: Cited in *Referate*. Ztschr. f. Krebsforsch., 1905, iii, 349.
- (39) MARCHAND: Cited by Gaylord and Clowes. Surg., Gyn. and Obst., 1906, ii, 648.
- (40) BOLOGNINO: Ztschr. f. Krebsforsch., 1908, vi, 261.
- (41) IBOTSON: Lancet, London, 1917, i, 224.
- (42) CZERNY: Ztschr. f. Krebsforsch., 1907, v, 27.
- (43) THEILHABER: Ztschr. f. Krebsforsch., 1913, xiii, 172.
- (44) COLEY: Am. Jour. Med. Sc., 1906, cxxxi, 385.
- (45) THEILHABER AND EDELBERG: Ztschr. f. Krebsforsch., 1913, xiii, 482.
- (46) CZERNY: Ztschr. f. Krebsforsch., 1909, vii, 294.
- (47) HEIDENHAIN: Cited by Czerny. Ztschr. f. Krebsforsch., 1909, vii, 294.
- (48) HANDLEY: Cited by Theilhaber and Edelberg: Ztschr. f. Krebsforsch., 1913, xiii, 478.
- (49) VIDAL: Travaux de la deuxième Conférence internationale pour l'étude du cancer, Paris, 1910, p. 302.
- (50) MUNDI: Amer. Jour. Obst., 1908, lvii, 403.
- (51) SCHWARZ: Virchows Arch. f. path. Anat., 1904, clxxv, 507.
- (52) GRAVERS AND PRICE: Amer. Jour. Obst., 1917, lxxv, 250.
- (53) SCHLIMPERT: Monatschr. f. Geburtsh. u. Gynäk., 1913, xxviii, 306.
- (54) SANGER: Verhandl. d. deutsch. Gesellsch. f. Chir., 1894, xxiii, 171.
- (55) BULLOCK AND ROHDENBURG: Jour. Med. Research, 1915-16, n.s., xxviii, 53.
- (56) CROSBIE: Brit. Med. Jour., 1899, i, 338.
- (57) HODENPYLE: Med. Rec., New York, 1910, lxxvii, 359.
- (58) BUTLIN: Brit. Med. Jour., 1892, ii, 1343.
- (59) PROCHOWNICK: Sitz. d. Geburtsh. Gesellsch. zu Hamburg, Berl. klin. Wehnschr., 1906, xlv, 323.
- (60) LITTAUER: Arch. f. Gynäk., 1890, lxxii, 294.
- (61) GROFE: Zentralbl. f. d. ges. Gynäk. u. Geburtsh., 1904, lxxii, 294.
- (62) LANGHANS: Beitr. z. Geburtsh. u. Gynäk., Berlin, 1901, v, 1.
- (63) HORMAN: Beitr. z. Geburtsh. u. Gynäk., Berlin, 1904, viii, 418.
- (64) KLOTZ: Zentralbl. f. Gynäk., 1896, xx, 841.

- (65) FRANKEL: Zentralbl. f. Gynäk., 1886, x, 771.
- (66) SIGG: Cor.-Bl. f. schweiz. Aerzte, 1891, xxi, 261.
- (67) KUTZNER: Zur Kaustik und Histogenese der Lymphosarcom, Diss. Inaug., Greifswald, 1889.
- (68) KLEEBLATT: München. med. Wehnschr., 1890, xxxvii, 107.
- (69) FLEISCHMAN: Monatschr. f. Geburtsh. u. Gynäk., 1903, xvii, 415.
- (70) LICK: Monatschr. f. Geburtsh. u. Gynäk., 1906, xx, 259.
- (71) SCHKARIN: Prag. med. Wehnschr., 1905, xxx, 509.
- (72) WALSCHÉ: Cited by Mohr. Therap. Monatsh., 1903, xvii, 553.
- (73) WEBB: Cited by Mohr. Therap. Monatsh., 1903, xvii, 553.
- (74) DE GAETANO: Gior. internat. d. sci. Medicine., 1903, xxv, 215.
- (75) BILLROTH: Prag. med. Wehnschr., 1893, xviii, 51.
- (76) MOHR: Therap. Monatsh., 1903, xvii, 553.
- (77) RQUX: Cited by Mohr. Therap. Monatsh., 1903, xvii, 553.
- (78) WIDMER: München. med. Wehnschr., 1907, liv, 619.
- (79) HUTCHINSON: Cited by Mohr. Therap. Monatsh., 1903, xvii, 553.
- (80) PAGET: Cited by Mohr. Therap. Monatsh., 1903, xvii, 553.
- (81) BILLROTH: Prag. med. Wehnschr., 1893, xviii, 51.
- (82) JORDAN: Ztschr. f. Geburtsh. u. Gynäk., 1901, xl, 300.
- (83) VON FRANQUE: Ztschr. f. Geburtsh. u. Gynäk., 1908, xlix, 63.
- (84) GOULD: Clin. Jour., London, 1902, xx, 96.
- (85) LOMER: Ztschr. f. Geburtsh. u. Gynäk., 1903, i, 305.
- (86) CLARKE: Clin. Jour., London, 1906, xvii, 264.
- (87) CHROBAK: Wien. klin. Wehnschr., 1905, xvii, 964.
- (88) KOSTER: Ztschr. f. Augenh., 1908, xix, 28.
- (89) WARRING: Tr. Clin. Soc., London, 1905, xxxviii, 210.
- (90) LOEFFLER: Deutsch. med. Wehnschr., 1900, xxvi, 725.
- (91) RIFFEL: Weiter pathologische studien über Schwindsucht und Krebs, Frankfort a Main, 1901.
- (92) WELLS: Jour. Am. Med. Assn., 1901, lii, 1731.
- (93) FRIEDMAN: Jour. Am. Med. Assn., 1901, xxxvi, 1309.
- (94) GRAHAM: Tr. Path. Soc., Chicago, vii, 8.
- (95) RICHARDSON: Tr. Am. Surg. Assn., 1898, xvi, 309.
- (96) PROESCHER: Jour. Am. Med. Assn., 1907, xlviii, 480.
- (97) PERRIN: Rev. méd. de la Suisse romande., 1891, xi, 195.
- (98) STEUDEL: Beitr. z. klin. Chir., 1899, xxiii, 408.
- (99) LINDER: Beitr. z. klin. Chir., 1900, xxvi, 462.
- (100) COLEY: Am. Jour. Med. Sc., 1893, cv, 487.
- (101) RANDOLPH: Am. Jour. Med. Sc., 1905, cxxxix, 74.
- (102) FLEISCHMANN: Wien. med. Wehnschr., 1908, xxi, 1492.
- (103) HALL: Arch. Int. Med., 1908, ii, 360.
- (104) SAAR: Beitr. z. klin. Chir., 1908, lvii, 231.
- (105) PETERSON AND COLMERS: Beitr. z. klin. Chir., 1904, xliii, 159.
- (106) WILLIAMS: Twentieth Century Practise, 1898, xvii, 394.
- (107) VON HANSEMAN: Cited by Mohr. Therap. Monatsh., 1903, xvii, 553.
Mikroskopische diagnose bösartigen Geschwulste, Berlin, 1902.
- (108) MARTIN: Deutsch. med. Wehnschr., 1908, xxxiv, 89.
- (109) GERSTER: Cited by Coley. Am. Jour. Med. Sc., 1893, cv, 487.

- (110) HENDRY: New Zealand Med. Jour., 1903, iii, 92.
- (111) CLELAND: Australasian Med. Gaz., 1909, xxviii, 301.
- (112) WARTHIN AND SPITZLEY: Med. News, London, 1901, lxxix, 443.
- (113) GUINARD: Bull. et mém. Soc. de chir. de Paris, 1904, n.s., xxx, 29.
- (114) EISSEMENGE: Wien. klin. Wchnschr., 1893, vi, 939.
- (115) JOHR: Zentralbl. f. Gynäk., 1894, xviii, 545.
- (116) RIESEL: Arb. a. d. Path. Inst., Leipzig, 1903, i, 104.
- (117) KAPOSÍ: Beitr. z. klin. Chir., 1901, xxx, 139.
- (118) BEATSON: Brit. Med. Jour., 1896, i, 1386.
- (119) LILIENTHAL: Internat. Jour. Surg., 1913, xxvi, 156.
- (120) MARTIN: Bull. méd., Paris, 1903, xvii, 727.
- (121) HANDLEY: Brit. Med. Jour., 1909, i, 582.
- (122) GOULD: Lancet, London, 1910, ii, 1672.
- (123) POWERS: Lancet, London, 1899, i, 583. St. Bartholomew Hosp. Rep., 1898, xxxiv, 239.
- (124) FISCHER: Cited by McConnell. Internal. Clin., 1910, 20th Series, ii, p. 98.
- (125) LADINSKI: Surg., Gyn., and Obst., 1915, xx, 325.
- (126) GAYLORD AND SIMPSON: Jour. Cancer Research 1916, i, 379.

EXPERIMENTAL "CARCINOMATA" OF ANIMALS AND THEIR RELATION TO TRUE MALIGNANT TUMORS

F. D. BULLOCK AND G. L. ROHDENBURG

*From Columbia University, George Crocker Special Research Fund, F. C. Wood,
Director*

Received for publication, August 14, 1917

Since Fischer (1) demonstrated a method of producing lesions of the skin of rabbits morphologically resembling human epitheliomata, reports have been published from time to time announcing the artificial production of malignant tumors by one or another procedure, the more prominent being those of Fibiger (2), Stahr (3), and Yamagiwa and Ichikawa (4).

Fibiger believes that he has produced gastric cancers in rats by feeding the animals with cockroaches, the host of the nematode worm *spiroptera*. After ingestion of the cockroach by the rat, the worms gain entrance to the tissues of the stomach, and set up in the squamous epithelium a proliferative process which may assume the appearance of a squamous carcinoma. Stahr asserts that he has caused epitheliomata of the tongue in rats by feeding the animals with oats, and Yamagiwa and Ichikawa have reported success in arousing malignant changes in the epithelium of the ear of rabbits by painting the surface repeatedly with coal-tar.

In a previous publication (5), we have shown that different varieties of epithelium react differently to the same irritant, and that the degree and extent of epithelial proliferation, the agent employed being constant, depends upon the regenerative power of the tissue and the area of the epithelial surface in contact with the irritating material. It was also pointed out that when the action of the irritant ceases there is a gradual subsidence of

the proliferation, followed by destruction and elimination of the newly formed tissue.

An analysis of the several reports mentioned reveals a number of assertions common to each, which are at variance with the recognized laws of malignant tumor growth, while, at the same time, it brings out points which cast a certain amount of doubt upon the authors' interpretation of their results.

1. For example, the age of the animal is a negligible factor in the incidence of these experimental lesions, young animals, both rats and rabbits, being almost as susceptible as old ones; hence, if we accept these tumors as malignant neoplasms, we must modify the current view concerning a "cancer age," which has hitherto been held applicable to animals as well as to man. We must conclude, in other words, that rats and rabbits differ in this respect from man, and from the mouse, in which Murray (6), Slye (7), and others have shown that age is a very important factor for the origin of carcinoma.

2. The tumors originate in tissues possessing a high grade of proliferative activity, and certain of them are restricted more or less to these tissues. Thus those described by Fibiger are confined to the large cephalic region of the rat stomach which is lined by stratified squamous epithelium; and although they invade the submucosa there is no involvement of the muscularis, and the glandular portion of the organ always remains free. The lesions described by Stahr and by Yamagiwa and Ichikawa also arise in tissues possessing a high regenerative power, the stratified squamous epithelium of the dorsum of the rat's tongue in the region of the papilla vallata, in the case of the former investigator, and the epidermis of the rabbit's ear in the case of the two latter.

3. The extent of growth depends either upon the number of foci of irritation, or upon the number of times that the extrinsic irritant has been applied and the area of the epithelial surface involved. Thus Fibiger found that the size of the gastric lesions generally depends upon the number of worms present, and the other investigators showed that the extent of proliferation depends upon the area of epithelium affected by the irritant.

4. The authors, excepting Yamagiwa and Ichikawa, who found that their lesions often increased in size after irritation had been discontinued, do not state definitely whether continuous irritation is necessary for continuous growth, and whether a type of proliferation can be inaugurated that no longer depends upon the presence of the irritant; yet it is of great importance, from a diagnostic standpoint, to know the fate of these lesions after withdrawal of the extrinsic irritant. Even Yamagiwa and Ichikawa, however, have recorded the fact that one of their assumed carcinomata "merkwürdige Weise" receded spontaneously after the discontinuation of the irritation.

5. So far as we are aware, transplantation of these lesions either is not mentioned, or is said to have been unsuccessful. Again, the presence of metastatic growths described by two of the investigators remains, after all, unproved, as the appearances pictured might be the result of chronic irritation of the lymph-nodes, or as in one of Fibiger's cuts, a heterotopic epithelial area like that sometimes seen in the peritoneal nodes of man. Furthermore, the latter author has not differentiated his pulmonary metastasis from a form of bronchopneumonia which has been described in rats, and is characterized by the presence of squamous epithelium through metaplasia of the epithelium of the bronchus. Figure 14 represents this condition, as observed by us.

The object of this paper is to place on record the results of certain of our own experiments on chronic irritation, undertaken with the view of reproducing, through means other than those employed by the various authors quoted, such lesions as they have described, in order to ascertain whether these might not be interpreted as hyper-regenerative processes rather than as true carcinomata.

In the description of our experiments upon the rat stomach (8), it will be shown that the squamous epithelium of this organ possesses a high regenerative power, and reacts to irritants in very much the same manner as does the epidermis. Although we were unable to duplicate exactly the results obtained by Fibiger, certain localized lesions were produced simulating very completely the lesions caused by the nematodes, and suggesting

the possibility of what might result from a more delicate form of irritation.

The forms of irritation employed were mechanical, chemical, and a combination of the two.¹

Mechanical irritation was produced by balls composed of celluloid covered with spines of pig bristles, which were introduced into the normal stomach by operation, and suspended by a silk thread from the wall of the squamous region near the incision. Other balls made of cork, armed with pin points, and covered with celluloid and an outer coating of keratin for protection against the digestive action of the gastric juice, were introduced so as to lie free in the cavity of the organ; these were about the size of a pea, although a few larger ones were used. At irregular intervals of from ten to one hundred and fifty days, the animals were killed and the stomach examined. In several instances, however, the balls were removed at the end of three months and replaced by larger ones, which were allowed to remain until the death of the animals, several months later.

Since the lesions produced by these irritants showed a general similarity, varying merely in degree, only the most striking will be described.

The reaction of the squamous epithelium to spinous balls suspended from the wall consists of irregular polypoid growths situated at the site of the gastrotomy wound, the largest of which (fig. 1), measuring 2 cm. in length, was observed thirty-two days after the operation. Microscopically, the reaction is characterized by hypertrophy of the epithelium with extensive production of keratin, and some proliferation and downgrowth of epithelial cells, restricted, however, by a basement membrane and localized in the mucosa. The stomach is thrown into several large folds forming polypoid projections of the mucosa. The reaction is probably due in large part to the fact that the

¹ A total of 259 rats were used for the experiments upon the stomach and tongue. For mechanical irritation of the stomach by spinous balls, 84 rats were employed; 14 of these had the balls suspended by a silk thread, while 70 had the balls lying free in the stomach chamber. For chemical and mechanico-chemical irritation of the stomach 132 rats were necessary, while 33 animals were used in the tongue experiments and 10 for injection of the salivary glands.

gastrotomy wound was closed by a purse string suture, the suspended ball playing but a subsidiary rôle, since somewhat similar pictures were produced by simply pleating the stomach, without incision.

The lesions associated with balls lying free in the stomach are confined chiefly to the glandular portions of the organ, although papillary ulcers, generally multiple, have sometimes been observed in the squamous part (fig. 1). Such ulcers are not infrequent in the stomachs of nonoperated rats (fig. 2), and as they were not a common occurrence in the experimental animals it seems hardly permissible to attribute their presence in each case to the action of the balls, although it is not improbable that in a few cases these were the causative factor.

Histologically, the mucosa presents an irregular papillomatous structure with here and there a loss of epithelium, with or without partial or complete healing of the ulcerated area. The ulcers are generally superficial, involving only the mucosa, and are covered by bacteria, moulds, yeast, leucocytes, and epithelial squames. The epithelium shows marked hypertrophy and an overproduction of keratin, and the formation of finger-like processes of cells which extend into the mucosa, often reaching to the muscularis mucosa (fig. 3).

The general reaction of the stomach following the introduction of free spinous balls consists of an hypertrophy, most marked in the glandular region, where the walls may be 3 to 4 mm. thick; with the augmentation in thickness there occurs a great increase in the production of mucus. Multiple ulcers, both gross and microscopical, are generally still present in this region, although occasionally they have completely healed. In exceptional cases the stomach shows dilatation without compensatory hypertrophy.

When the incision made to introduce the ball involves the glandular region, the most marked lesions are noted at the site of the cut; here there occurs an irregular downgrowth of the hypertrophic epithelium which covers the area of denudation, forming cysts of varying number and size. There is considerable hypertrophy and dilatation of the tubules and an

increase in the size of the cells, particularly of those tubules lining the cavity. There is also a great increment in the number of mucous elements, with an absence or paucity of parietal cells, giving the gastric epithelium the appearance of intestine. When healing is complete, the epithelium which has grown down into the depths becomes isolated from the surface epithelium, producing a picture resembling a cystadenoma (fig. 4). In or bordering the area of injury, small areas of connective tissue of the stratum proprium are sometimes converted into osteoid tissue. The stomach walls show a varying degree of inflammation, and collections of lymphoid cells are occasionally observed in the submucosa or beneath the serosa. Small cystic dilatations of the tubules and earlike processes of mucosa projecting into the cavity of the stomach are sometimes observed in other regions of the organ. When the gastrotomy incision is confined to the squamous portion of the stomach, similar but less extensive lesions are found in the glandular region at the site or sites of injury by the ball.

The methods employed for chemical or mechanico-chemical irritation of the stomach were (a) direct application of an irritant to the epithelium of the stomach, and (b) injections of the irritant into the wall of the organ after gastrotomy. Scharlach R and pine tar, either in their original state or dissolved in oil or ether, were used as the chemical irritants, the stomachs being examined from the seventh to the fifteenth day after the infliction of the injury.

The reaction of the squamous epithelium of the viscus to cotton wicks soaked in Scharlach R oil or pine tar oil and passed through the stomach wall, resembles that of the epidermis when it is subjected to a similar form of irritation. The thickened epithelium grows along the surface of the wick to reach the outer or serous coat of the organ; while at or bordering the site where the epithelium turns outward to follow the course of the wick there is a more marked grade of hypertrophy and hyperkeratinization with atypical proliferation of the underlying cells (fig. 5). Processes of cells from the inverted epithelium reach out into the connective tissue toward similar prolongations from

the overlying epithelial layer. The stomach walls are thickened and present an inflammatory reaction.

When single pieces of rubber sponge, either impregnated with Scharlach R powder or covered with pine tar, are introduced into the stomach, they give rise to hypertrophy and proliferation of the squamous epithelium about the line of suture which closes the gastrotomy opening. The most striking reaction, observed thirteen days after the introduction of a piece of sponge impregnated with Scharlach R powder, is characterized by extreme localized hypertrophy of the epithelium with the production of thick blunt processes of cells, sometimes branching, which extend deeply into the stomach wall and reach below the normal site of the muscularis mucosa. In cross section, these processes are represented by rounded nests of cells showing central cornification. A distinct basement membrane limits these cells in their growth (fig. 6); still, though localized and restricted in its growth, the lesion bears some resemblance to an epithelioma, and may be compared with the larger lesions produced by parasites.

The results of a single injection of Scharlach R or of pine tar in oil or ether into the stomach, consist chiefly of a moderate grade of epithelial hypertrophy and the formation of epithelial cysts about the injected material.

Twice repeated injections of pine tar at intervals of seven days give rise, six days after the last injection, where the injected material comes into intimate contact with the surface epithelium, to a reaction characterized by hypertrophy of the epithelium with the production of thick processes extending below the muscularis mucosa. Here the epithelium tends to surround the injected material. There is an increase in keratin formation and an inflammatory edema of the mucosa (fig. 7). Repeated injection of the irritant may also cause papillomatous tumors, with or without ulceration, such as have already been described.

Attempts to inaugurate a proliferative process by the introduction of a rubber sponge infiltrated with Scharlach R powder, followed ten days later by the injection into this region of one or the other irritant into the wall of the stomach, result in a con-

siderable proliferation of the epithelium chiefly confined to the mucosa in the region of the suture line. Downgrowth of the epithelium about the injected material reaching below the muscularis mucosa (fig. 8) may also occur.

Injections of the irritant into the stomach wall from without the body of the rat, at the gastric edges of a gastrostomy opening, result in papillomatous tumors or ulcers showing a general resemblance to those produced by repeated injections made without opening the viscus. Owing to the impossibility of keeping rats subjected to gastrostomy alive for any considerable period (over fourteen days) the injections could not be repeated more than once.

The following conclusions may be drawn from the results of these experiments. The epithelium of the rat stomach, both squamous and glandular, possesses a high regenerative power and shows an active response to irritation. Where tissue resistance is reduced and the irritation is continued, the epithelium may invade to some depth the wall of the stomach. Lesions resembling cystadenomata may be produced in the glandular region of the stomach by simple mechanical irritation, and papillomatous tumors and lesions suggesting epitheliomata may be produced in the squamous region by chemical or mechanical means.

Now if we assume that the parasite described by Fibiger elaborates constantly an irritating secretion which acts continuously upon a wide area of epithelium, and that the epithelium responds to the stimulus by active proliferation; and if we grant the power of locomotion to the parasite, the zone of epithelial reaction is increased and new foci of irritation are produced by each progressive movement of the worm. The parasite, acting simply as a foreign body in contact with the epithelium, no doubt plays a minor part in the reaction. It thus irritates the epithelium of the stomach in a manner which we cannot duplicate; that is, by constant irritation over an ever increasing area of epithelium, upon which depends the degree and extent of epithelial proliferation in a specific tissue.

In the presence of an irritant in the tissues, atypical proliferation and invasive growth of the epithelium are in themselves doubtful criteria upon which to base a judgment of malignancy. Since marked atypical proliferation results from such relatively crude experimental methods of irritation as those described in preceding paragraphs, the more extensive reactions caused by the parasite with its refined methods are readily explainable, without malignant properties necessarily having been bestowed upon the epithelial cell.

The papillomatous tumors of the rat's tongue described by Stahr which arose after the repeated ingestion of oats, were associated with the presence of fine hairs derived from the husk of the grain; these hairs, which were incorporated in the growths, Stahr regarded as the exciting cause of the epithelial proliferation.

In our experiments upon the tongue of the rat, both mechanical and chemical irritation were employed. The mechanical method consisted of inserting into the tongue fine hairs of bearded wheat or foxglove. Several punctures were made by a needle in the dorsum of the tongue in the region of the papilla vallata, and the hairs were introduced into the openings in such a manner that the spines of the hair pointed outward, thus serving as barbs to prevent the hair from coming out. After the hairs had been thrust into the tongue for a short distance their ends were cut off close to the dorsal surface of the organ.

This form of irritation resulted in ulceration about the hairs, a considerable grade of hypertrophy of the lingual epithelium, and a downgrowth of the epithelium to surround the foreign body.

The method of chemical irritation elected was the injection into the organ, in the region of the papilla vallata, of Scharlach R dissolved in oil or ether, a hypodermic syringe with an L-shaped needle being used to inject the irritant. Little epithelial reaction followed the injection of Scharlach R in oil, owing, no doubt, to the difficulty of obtaining contact between the epithelium and the dye. However, Scharlach R dissolved in ether produced considerable ulceration, with hypertrophy and downgrowth of epithelium to wall off the area of ulceration, and meta-

plasia of the serous and mucous glands of the region involved. The gland acini were converted into squamous epithelium showing central cornification, and aggregations of these transformed gland elements presented the picture of a rather benign form of epithelioma. The stroma of the gland was edematous and showed active proliferation (fig. 9).

In order to determine whether the change in type of the gland epithelium was a true metaplasia or an extension downward into the acini of the proliferating surface epithelium of the tongue, the irritant was injected into the parotid and submaxillary glands through an incision in the skin at some distance from the site of the gland. The results of injection into these glands were even more striking than those observed in the lingual glands, and indicate that the transformation is a true metaplasia (fig. 10).

It would be wrong to conclude from these experiments that the lingual epithelium does not react to irritants to the same degree and extent as does the gastric epithelium, for technical errors, due partly to the porous nature of the tissues of the tongue, probably account for the difference. The interesting feature of the experiments is a metaplasia of the gland elements, which might be mistaken for epithelioma.

In a study of the Scharlach R reaction, we have pointed out that the reaction of the skin of rabbits caused by a single injection of the dye, reaches its height of proliferative activity about the eighth day following the injection. It was thought that if injections of Scharlach R oil were made at the same site at intervals of seven or eight days, so that one injection followed another at a time when the epithelial reaction resulting from the former injection was most active, a summation of effects might be produced. The increased difficulty of securing contact between the dye and the epithelium after the first injection was realized before undertaking the experiment.

The characteristic lesion produced in the skin of the rabbit by a single injection of Scharlach R oil consists of hyperplasia and downgrowth of the epithelium of the hair follicles, with an overproduction of keratin. In its downward course, the pro-

liferating epithelium surrounds the injected material and sends off from its periphery numerous strands of cells which extend into the depths to surround the oil droplets which they encounter, or reach out laterally into the tissues in an attempt to join similar processes from adjacent follicles. The surface epithelium may or may not participate. Cyst formation is the usual outcome of the active process.

One hundred and eight rabbits were used in this experiment, the injections being made into the skin of the buttocks or the back. These animals received from three to sixteen injections during a period of four months. In 59 rabbits the experimental area was excised and submitted to microscopical examination. In the remainder, the lesions gradually subsided and eventually disappeared clinically, though the animals were kept under observation for months after the experiment.

Three general types of reaction result from repeated injections of the dye. (a) When the hair follicles are chiefly involved in the proliferative process, a lesion is produced which, although often of greater degree and extent, resembles that caused by a single injection. Cyst formation is the characteristic result (fig. 11). (b) When the epidermis and the upper part of the hair follicles are chiefly concerned, owing to complete or partial destruction of the follicles, the lesion somewhat resembles that produced in the stomach by repeated injections of the dye. There is marked hypertrophy of the epidermis, with processes projecting from it into the connective tissue. Nests of epithelial cells showing central keratinization are observed; cysts are seldom encountered (fig. 12). (c) When both hair follicles and epidermis are involved, one or the other predominating, a reaction is produced which assumes the features of both (a) and (b).

In general, it may be stated that when the hair follicles assume the chief rôle in the proliferative process, cyst formation is the characteristic feature of the reaction, whereas, when the epidermis takes that part, papillary hypertrophy without cyst formation results.

A lesion worthy of separate description, since it differs from the usual type and bears a more striking resemblance to epi-

thelioma, was observed in a rabbit subjected to seven injections of the dye. The tumor, a warty growth showing a superficial area of necrosis, was removed from the animal fifty-five days after the first, and seven days after the last injection. The animal died a few days after the operation.

On microscopical examination, the hair follicles are found converted into parallel columns or strands of cells packed closely together and often anastomosing one with the other. These columns and strands are continuous above and below with large irregular columns or clumps of cells showing central cornification or beginning cyst formation. From the deeper lying clumps of cells, strands emerge to surround the oil droplets in the neighborhood. The smaller columns of cells often show a moderate grade of central keratinization. Numerous mitotic figures are observed in all parts of the lesion, which is well vascularized. The tendency of the epithelium of the hair follicles to form cysts, a characteristic of the usual reaction caused by injections of Scharlach R, is lacking or in abeyance (fig. 13). The overlying skin is ulcerated and there is a deep area of ulceration along the track of the needle. Although this lesion shows considerable deviation from the usual type, and cannot be distinguished from a true epithelioma as observed in man, we do not believe we are justified in calling it a malignant tumor. To us it appears to present only an expression of individual variation in the reaction of tissues to prolonged irritation.

Yamagiwa and Ichikawa base their claim to the production of epitheliomata on the rabbit's ear upon lesions not much more characteristic of epithelioma than the one we have just described. Although they state that they have observed epithelial cells in the blood-vessels, and metastatic deposits in the lymph-nodes, these observers by no means definitely prove their claim. The cuts of the lymph-nodes resemble the endothelial hyperplasia accompanying chronic irritation more than metastases of epithelial cells.

It is well known that benign tumors, such as chondromata, metastasize, and it is not difficult to conceive that with repeated trauma the walls of blood- or lymph-vessels may be injured,

allowing the epithelial cells to enter and be transported (as are chorionic masses) to another region of the body where they continue to grow for a time. In an unpublished experiment upon autologous grafts of proliferating ear epithelium, the proliferation being induced by single injections of Scharlach R oil, we have found such grafts surviving for fifty-five days in a foreign locality.

SUMMARY

Papillary and polypoid tumors, papillary ulcers of the squamous portion of the rat's stomach, and lesions of the glandular portion suggesting cystadenomata, are readily produced by simple mechanical injury.

Chemical or mechanico-chemical irritation of the squamous epithelium of the rat's stomach results in localized papillary tumors suggesting the canceroid growths produced by nematode worms.

The injection of Scharlach R in the region of the papilla vallata of the rat's tongue gives rise to a metaplasia of the serous and mucous glands of this region, with the production of lesions simulating a benign epithelial growth. Similar changes are produced in the parotid and submaxillary glands of rats by the same means.

Repeated injections of Scharlach R in oil into the skin of the buttocks of rabbits may cause lesions indistinguishable morphologically from human epitheliomata.

In view of the results of mechanical and chemical irritation of epithelium, as described in this paper, extreme caution should be observed before reports of the production of cancer by artificial means are accepted. In irritation tumors, atypical proliferation and invasive growth, even when extensive, are doubtful criteria upon which to base a judgment of malignancy; and in the absence of continued growth after the action of the extrinsic irritant has ceased, experimental tumors should not be classed as malignant, however close be the morphological resemblance. The fact that the lesions reported by the various observers quoted are produced as readily in young as in old animals, and

that apparently they do not possess the power of continuous growth upon transplantation into animals of the same species, increases the doubt regarding their malignant properties.

All these facts point to the conclusion already deduced from clinical observation, that age, organ specificity, and congenital defects play an important and, in many cases, a decisive rôle in the determination of the origin of cancer, and that irritation alone is an insufficient factor.

REFERENCES

- (1) FISCHER: Verhandl. d. deutsch. path. Gesellsch., 1906, x, 20-22.
- (2) FIBIGER: Ztschr. f. Krebsforsch., 1914, xiv, 295.
- (3) STAHR: Zentralbl. f. allg. Path. u. path. Anat., 1903, xiv, 4.
- (4) YAMAGIWA AND ICHIKAWA: Mitt. a. d. med. Fakult. d. k. Univ. zu Tokyo, 1915-16, xv, 295-344; 1917, xvii, 19; Jour. Cancer Research, 1918, iii, 1.
- (5) BULLOCK AND ROHDENBURG: Jour. Med. Research, 1915, xxxiii, 53.
- (6) MURRAY: Proc. Roy. Soc., Series B., 1911-12, lxxxiv, 42.
- (7) SLYE: Jour. Cancer Research, 1916, i, 479.
- (8) BULLOCK AND ROHDENBURG: Proc. Soc. Exper. Biol. and Med., 1915, xii, 161.

PLATE I

Fig. 1. Gross appearance of the polypoid ulcers produced in the squamous portion of the rat stomach by spine balls.

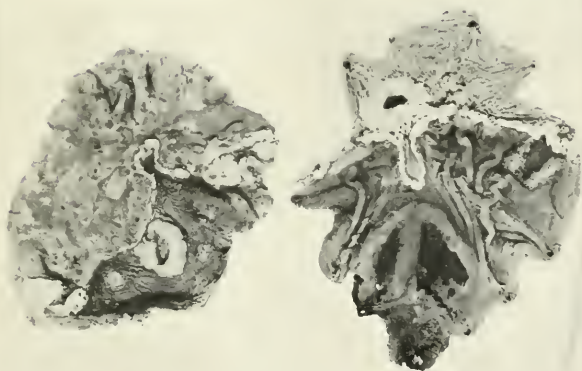
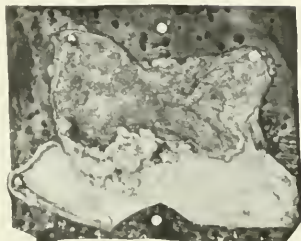


PLATE 2

Fig. 2. Spontaneous ulcers of the rat stomach, squamous portion and duodenum.

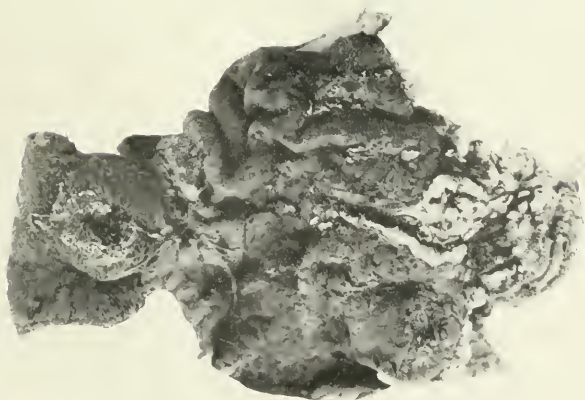
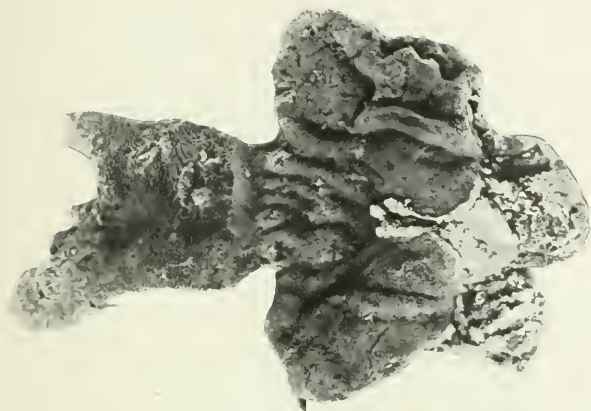
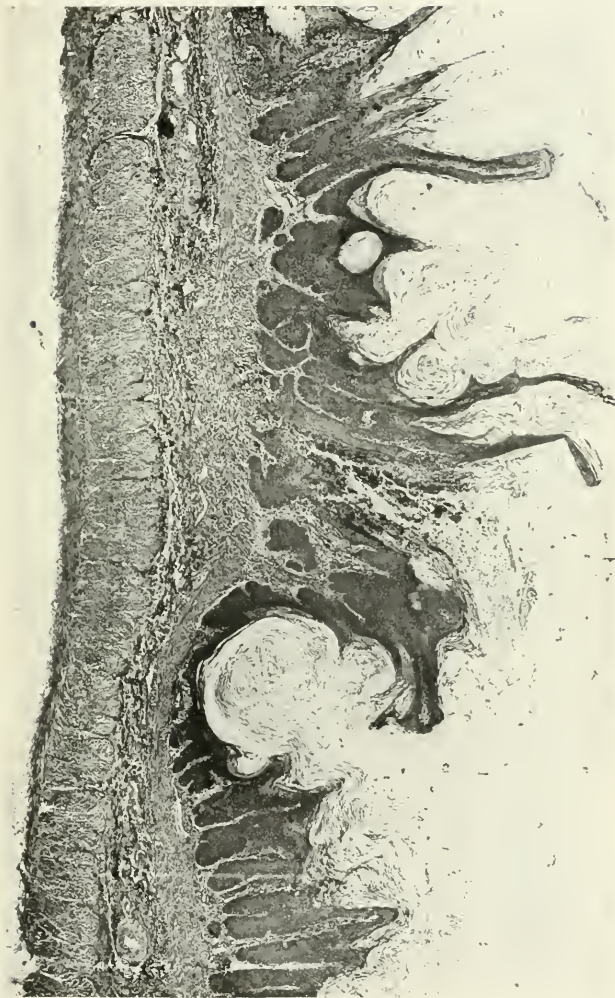


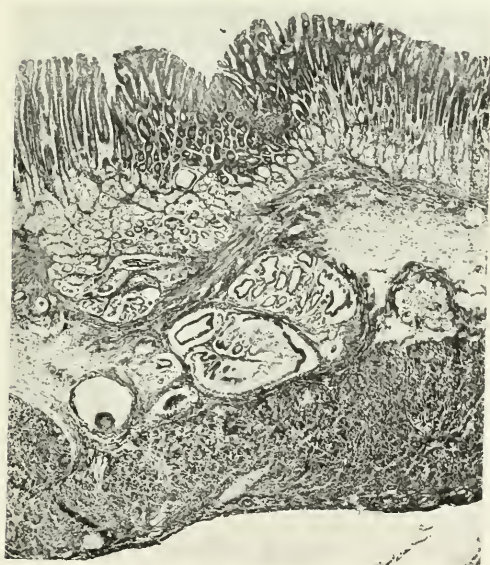
PLATE 3

Fig. 3. Reaction of the rat stomach after introduction of spine ball.



PLATES 4 AND 5

Fig. 4. Healed ulcers (ulcer produced by spine ball) of stomach resembling cystadenoma, showing various types.



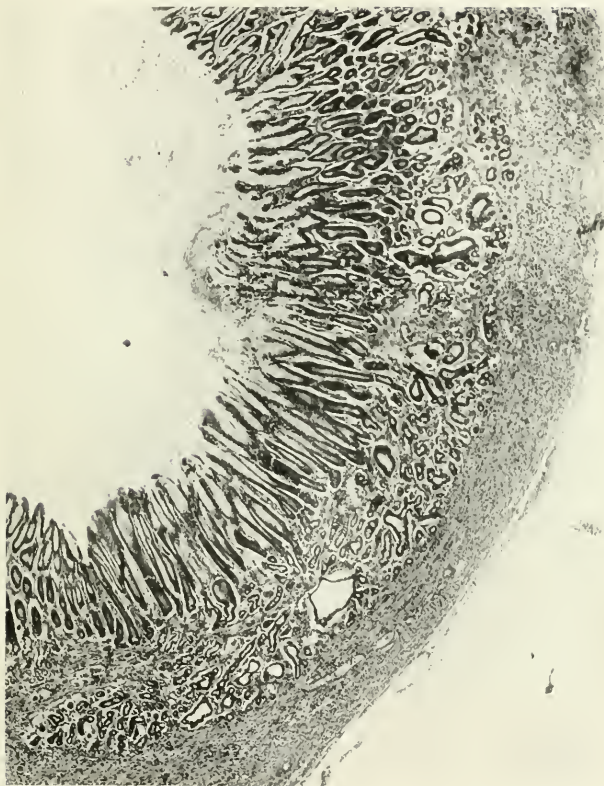


PLATE 6

Fig. 5. Marked keratinization and downgrowth of epithelium about wick.



PLATE 7

Fig. 6. Lesion produced by sponge saturated with Scharlach R powder, when introduced into the stomach of the rat.

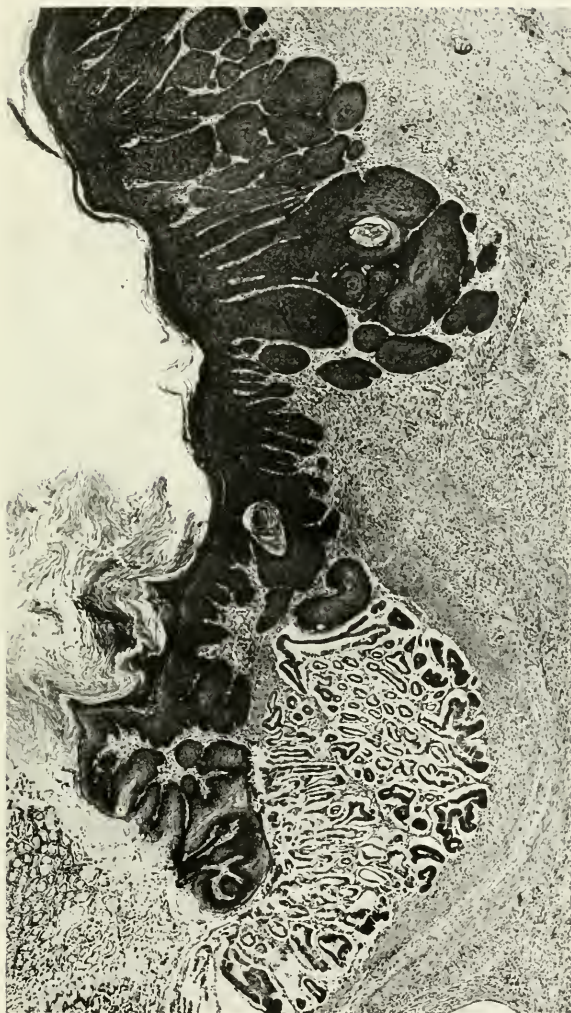


PLATE 8

Fig. 7. Reaction of the squamous epithelium of the stomach to injections of pine tar.

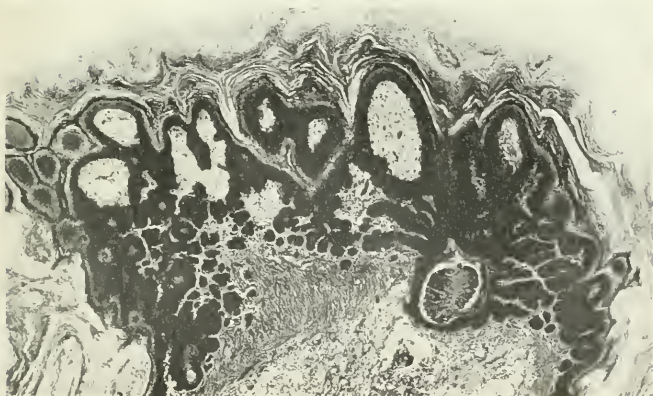
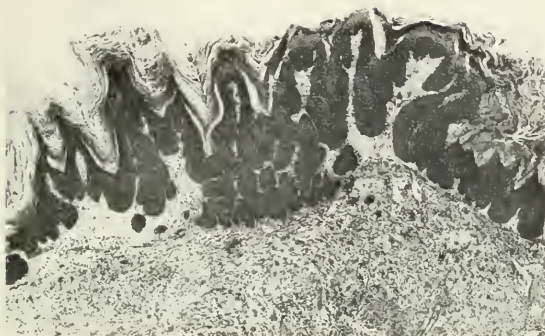


PLATE 9

Fig. 8. Downgrowth of epithelium about injected material (pine tar) following irritation with sponge saturated with Scharlach R.



PLATE 10

Fig. 9. Metaplasia of the serous glands of the rat tongue.

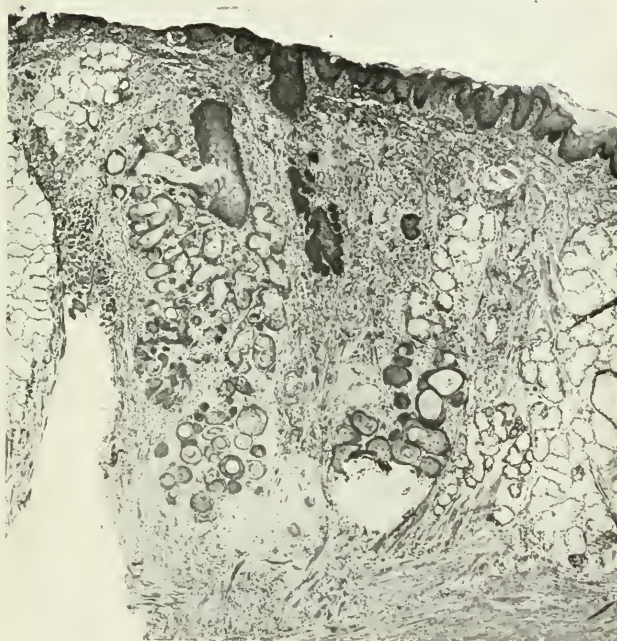
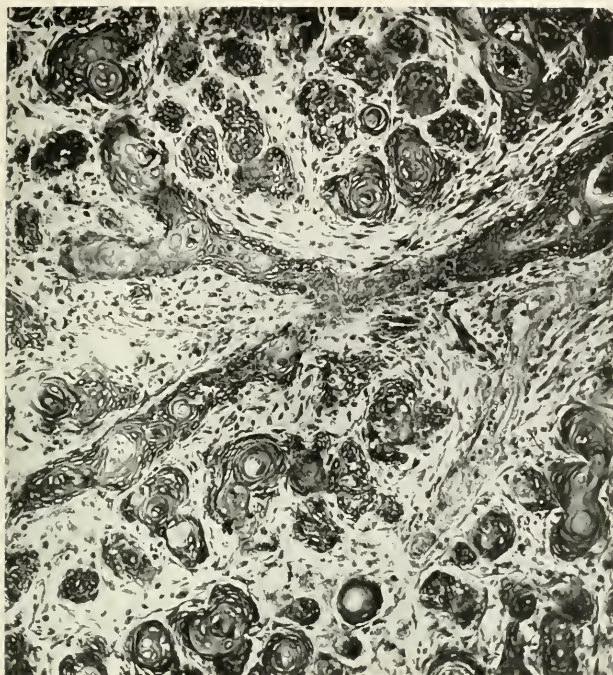


PLATE 11

Fig. 10. Different magnification of reaction in the submaxillary gland after injections of Scharlach R.



45

PLATE 12

Fig. 11. Type A of the Scharlach R reaction in the skin of the rabbit.



PLATE 13

Fig. 12. Type B of the Scharlach R reaction in the skin of the rabbit.



PLATE 14

Fig. 13. Unusual reaction of skin of rabbit after repeated injections of Scharlach R.

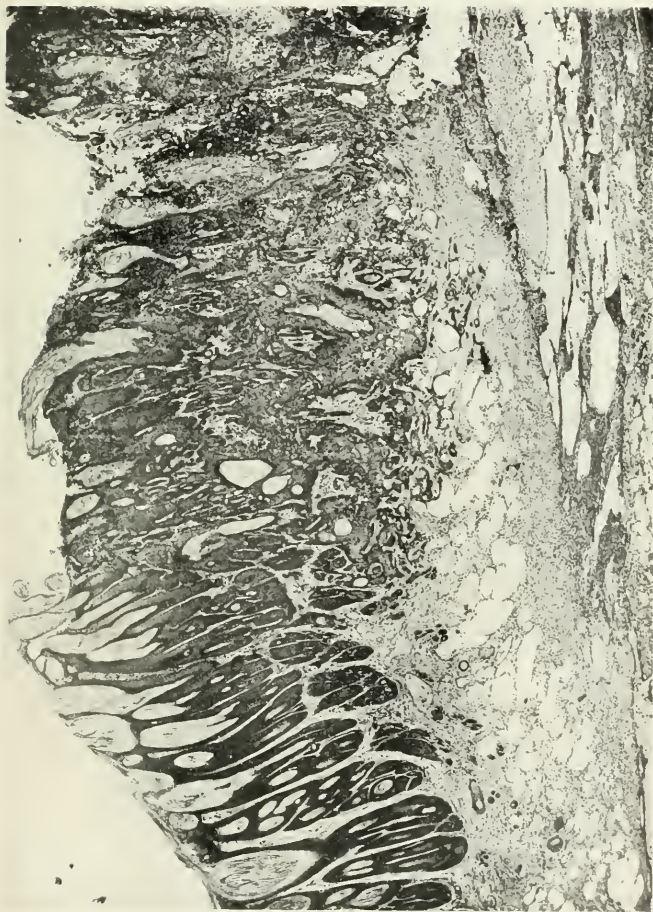
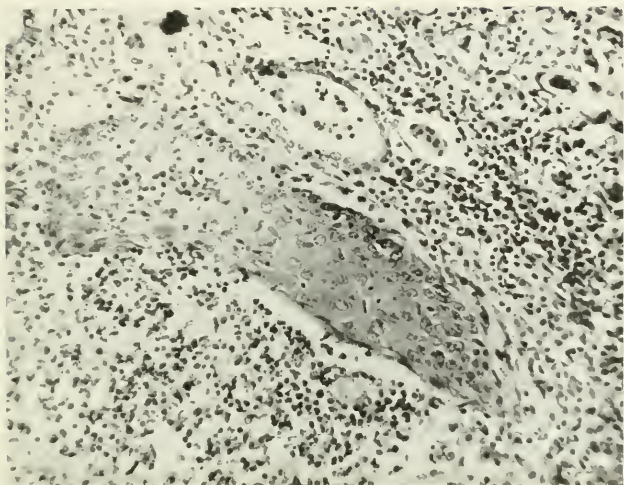


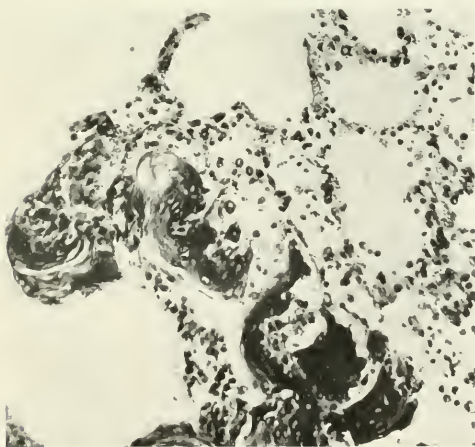
PLATE 15

Fig. 14. Metaplasia of the bronchial epithelium in the rat lung associated with bronchopneumonia. Note mitotic figures in "A."

F. D. BULLOCK AND G. L. RORDENBURG



A



B

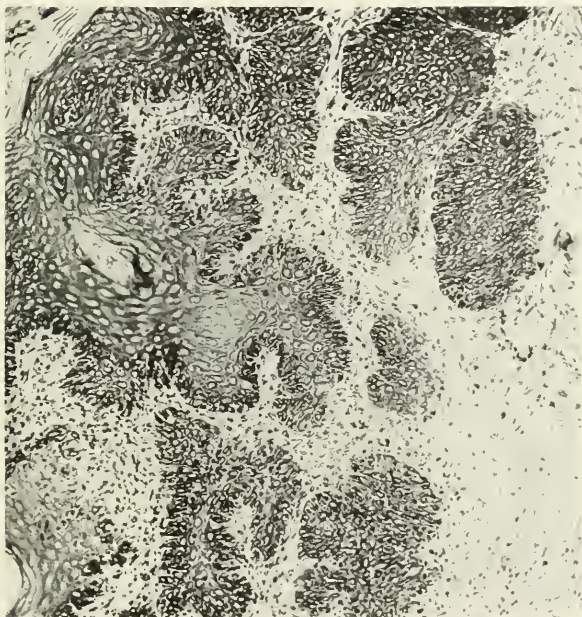
PLATE 16

Fig. 15. Proliferation induced in rabbit skin by parasitic skin infection. Compare with reaction induced by Scharlach R.



PLATE 17

Fig. 16. Reaction of squamous epithelium of stomach after injections of Scharlach R oil into edge of gastrostomy opening.



ADENOCARCINOMA OR ADENOMA OF THE LIVER IN A MOUSE

SHIGEMITSU ITAMI

*From Columbia University, George Crocker Special Research Fund, F. C. Wood,
Director*

Received for publication, August 18, 1917

Notwithstanding the great frequency of spontaneous mammary neoplasms in the mouse, few cases of primary hepatic tumor in this species have been reported. The first example, an adenoma, probably malignant, was described by Murray (1), while Slye (2) has recorded twenty-eight cases ranging from simple benign adenoma to true carcinoma, to say nothing of several others discussed in connection with her work on the inheritability of a tendency to develop neoplasms.

The following example, which is the first that has come under observation in this laboratory, is reported on account of the comparative rarity of primary growths in this organ.

The growth (fig. 1) was discovered in a female mouse from which a spontaneous adenocarcinoma of the mamma had been removed. The animal died forty-four days later, without recurrence. At the autopsy, a nodule was found in the left lobe of the liver near the free margin, which proved upon incision to be a white lenticular mass of which the greatest diameter was about 0.5 cm. It was sharply demarcated from the remainder of the liver, which was of the ordinary color. The lungs were free from secondary growths. As decomposition was beginning, transplantation was not attempted.

Microscopically, the tumor was composed of anastomosing cords of cells resembling liver cells, except that they were very large and much more deeply stained. The nuclei, which also exceeded the normal limits, were rich in chromatin, but no mi-

totic figures were discoverable. As in the normal liver, many cells contained two or more nuclei.

The cords of these elements, though no longer disposed in the manner characteristic of the normal liver, still suggested a radial arrangement about a central vein, thus forming an irregular imitation of the lobule. Between the cords or cells there were either lumina or blood-vessels which corresponded to the nor-

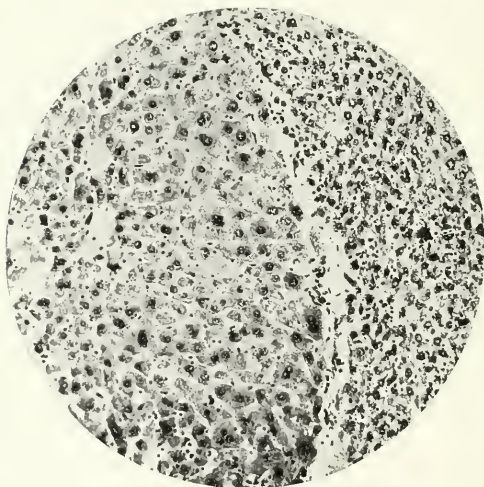


FIG. 1. ADENOCARCINOMA (?) OF THE LIVER. $\times 200$

mal capillary bile ducts and intralobular blood-vessels respectively. Even small bile ducts lined by low epithelial cells could be found, accompanied by the portal vein. In a few areas toward the center, the tumor showed a slight hemorrhage or necrosis.

The growth had no connective tissue capsule, yet invasion of the surrounding organ could not be demonstrated. About the margin, where the growth adjoined the normal liver, a few cords

of cells that had been flattened by expansive pressure formed a distinct boundary for the neoplasm. Careful study of serial sections showed that no gradual transition between the tumor and its normal surroundings, a condition which has been repeatedly observed and discussed in the human subject, could be demonstrated. On the contrary, there was the abrupt histological transition and the sharp demarcation described above. The remainder of the organ exhibited fatty changes.

The growth in question is certainly not a metastasis from a mammary carcinoma, but it is difficult to decide exactly what it is. The absence of mitotic figures, invasive growth, and metastasis, all indicate a benign neoplasm; yet, on the other hand, the abnormal size of the cells and their irregular staining, no less than the atypical arrangement, suggest malignancy. A positive diagnosis is, in fact, impossible, and the diagnosis must remain in doubt until such time as the possession of more accurate information permits a decision to be made.

REFERENCES

- (1) MURRAY, J. A.: Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 69.
- (2) SLYE, M.: Jour. Med. Research, 1915, xxxiii, 171. Jour. Cancer Research, 1916, i, 503. Ibid., 1917, ii, 213.

AN HEREDITARY TUMOR IN THE FRUIT FLY, DROSOPHILA

MARY B. STARK

From the Zoological Laboratory, Columbia University

Received for publication, September 6, 1917

In the fruit-fly *Drosophila melanogaster* (*ampelophila*) there have been found by various workers certain factors called "lethals," the observed effect of which is to kill the fly at some pre-adult stage. There was, however, little direct evidence to show how these factors produced their lethal effect until the discovery by C. B. Bridges of a lethal, lethal 7, which causes the development of one or several black granules within the body of the larvae (Bridges, *Genetics*, Jan., 1916). Bridges observed that in the cultures of lethal 7, about a quarter of the larvae showed these intense black "spots" and further that these larvae behaved very differently from normal larvae, frequently crawling out of the food and wandering about upon the walls of the culture bottle. These black-spotted larvae died in great numbers either upon the walls of the culture bottle or in the food. From the definite mode of occurrence of these black-spotted larvae throughout many and varied experiments made to test the mode of inheritance and the location of the factor involved in lethal 7, he concluded that these black-granuled larvae were the males having the lethal 7 factor, and that their death was probably connected with the development of the black bodies. The lethal 7 stock was turned over to the author to find out (1) the nature of the black body found in the larvae that die; (2) whether it is in itself the cause of death or only a by-product of the condition of the larvae; (3) whether by removal of the black body the life of the larva would be prolonged; (4) whether the transfer of the black body to normal larvae would cause their death; (5) the effects

of *x*-rays upon the black body; (6) in what tissues the black body develops.

To establish the inferred causal connection between the lethal factor of this stock and the black-spotted larvae, eggs which were laid by four females from the lethal 7 stock were isolated. The larvae from these eggs were examined for spots and those with spots were removed from the culture bottle so that it might be determined whether any with spots pupated and emerged as flies, and whether the number with spots corresponded with the number of flies killed by the lethal factor. The results are shown in the first division of table 1.

TABLE 1

NUMBER OF PAIR	NUMBER OF EGGS	NUMBER OF LARVAE	NUMBER OF LARVAE WITH SPOTS	NUMBER OF FLIES	PERCENTAGE THAT HATCHED
1	174	153	38	110	63.00
2	246	219	53	151	61.00
3	129	111	30	91	70.60
4	268	223	56	111	41.3
Total.....	817	706	177	463	56.67

<i>Control</i>					
1	316	258	0	246	77.00
2	266	217	0	203	76.80
Total.....	582	475	0	449	77.00

The total number of eggs picked out was 817, of which 706 hatched as larvae. All the larvae with spots, 177 in number, died before pupation. This number is nearly one-fourth the total number of flies and corresponds therefore to the number killed by the lethal factor. Lethal 7, like other lethals studied, is sex-linked, always killing one-half of the males. To determine whether the larvae with spots are males or not, hundreds of these have been fixed, sectioned and stained, and examined microscopically, and in every case it has been noted that the larva is a male, as should be the case if the hypothesis were correct. There is evidently the assumed causal relationship

between the lethal factor and the dark spot, because all the dark spotted larvae die, and these correspond in number to the



TEXT FIG. 1. TUMOR ARISING IN EMBRYONIC CELLS NEAR THE POSTERIOR END OF THE LARVA

number expected for lethal factors; and also because all the larvae with spots are males.

In the control experiment, eggs from two females that were sisters of lethal bearing females but not themselves lethal bear-

ing were isolated.¹ The results obtained are shown in the last division of table 1.

The total number of eggs picked out was 582, of which 475 hatched as larvae. Of this number, 449 emerged as flies, which is about 77 per cent of the total number of eggs laid, a normal output for this strain of flies. No larvae with spots were found and nearly all the larvae pupated and emerged as flies.

The examination of both longitudinal and cross sections of larvae with spots revealed the fact that the dark bodies are cellular growths somewhat resembling the tumors of vertebrates; they will be called tumors here in the sense that they are abnormal cellular overgrowths (text fig. 1). The cells near the center are large, polyhedral, spheroidal, or fusiform in shape. Pigment is present both within and outside the cells. Toward the periphery the cells become flattened and closely crowded together, forming laminated layers of pigment giving to the tumor the appearance of being encapsulated.

TIME OF DEATH

Just before death, the larvae with tumors become more or less restless and tend to crawl away from the food, if possible, and up the sides of the culture bottles, where they become more and more inactive and finally die. Some of these larvae in the restless stage were measured. Those of the same length were placed in the same culture bottles (table 2).

TABLE 2

LENGTH OF LARVAE	APPROXIMATE AGE OF LARVAE	NUMBER PICKED	TIME OF DEATH
<i>mm.</i>	<i>days</i>		
1.5-2.0	1 -1½	15	Within 15 hours
2.0-2.5	1½-2	23	Within 15 hours
2.5-3.0	2 -2½	20	Within 15 hours
3.0-3.5	2½-3	36	Within 15 hours
3.5-4.0	3 -3½	44	Within 15 hours
4.0-4.5	3½-4	32	Within 15 hours

¹ Because of linkage the white eyed females of this lethal 7 stock are known not to carry the lethal factor.

In every case it was noticed that the larvae of table 2, when placed upon food, neither bored into the food nor fed upon it. Death always occurred within twelve or fifteen hours, activity decreasing within six hours. Restlessness is apparently an evidence of oncoming death. It is also apparent that the larvae with the tumor die at varying ages. If the tumor appear quite late, i.e., not until the second or third day of the larval stage, death occurs just before pupation, i.e., about the end of the fourth day. If, on the other hand, the tumor appear on the first day, death occurs on or about the second. In many cases it has been noticed that the tumor is fully developed (i.e., has become black) in a day old larva, and that death has occurred on the

TABLE 3

LENGTH OF LARVAE	NUMBER WITHOUT TUMORS	NUMBER WITH LIGHT TUMORS WITHIN TWELVE HOURS
<i>mm.</i>		
1.5-2.0	10	2
2.0-2.5	12	3
2.5-3.0	13	1
3.0-3.5	30	8
3.5-4.0	25	5
4.0-4.5	20	4

second day. The tumor in the early stages of development has very little pigment and is therefore scarcely perceptible to the eye. Many larvae with light colored tumors have been isolated and it has been noticed that within twenty-four hours the tumor is quite brown in color and often before thirty-six hours it is black. The larvae recorded in table 3, varying in length from 1.5 to 4.5 mm., were without tumors. Tumors appeared in some of each of these classes within twelve hours.

All the tumors were light at first appearance and became brown in color within twenty-four hours and black within thirty-six hours. Rarely does death occur until the tumor is fully developed; i.e., until the tumor is black with pigment. The tumors of the larvae of table 2 were all black in color. There is evidently a relationship between the occurrence of death and the ripening of the tumor.

To determine whether there is any relationship between the occurrence of death and the size of the tumor, larvae were picked and measured. The larvae picked out for these measurements given in table 4 were larvae that had crawled away from the food to die. The results in this table show that nearly as many deaths occur among larvae from 2 to 3 mm. in length as among those from 4 to 4.8 mm. in length. More than half the larvae measured were between 3 and 4 mm. in length, while the other half is composed of the smaller larvae from 2 to 3 mm. in length and the larger larvae from 4 to 4.8 mm. in length.

In this table are indicated also the lengths, breadths, and approximate volumes of the tumors. From these measurements were computed the correlated variates and the correlations with the probable errors, as recorded in table 5.

The correlation of the length of the larvae to the number of tumors is 0.048 ± 0.065 ; this is a negligible correlation. The figures on plate I bear out the same fact. The enlargement is the same for all the drawings made and one of the smallest larvae drawn contained sixteen tumors (plate I, fig. 1). The correlation of the length of larva to length of tumor is 0.127 ± 0.049 ; this is scarcely a significant correlation. The correlation of the length of larva to the breadth of tumor is 0.169 ± 0.048 . This is likewise a correlation of doubtful significance. The correlation of length of larva and volume of tumor or of tumors, 0.094 ± 0.065 , is negligible.

There is, therefore, no relation between the size of the larvae and the number of tumors, and only a doubtful relation between the size of the larvae and the size of the tumors. The correlation between the number of tumors and the length of tumors is -0.390 ± 0.042 ; that between the number of tumors and the breadth of tumors is -0.388 ± 0.043 ; that between the length of tumor and the breadth of tumor is 0.748 ± 0.022 . The last three correlations are, then, all significant, and show, first, that whenever the tumors are numerous they are small, and second, that the shape of the tumors is relatively constant.

In table 6 are shown the physical constants of distribution. The mean length of the larva is 3.742 ± 0.058 ; its standard

TABLE 4

LENGTH OF LARVA	LENGTH OF TUMOR	BREADTH OF TUMOR	VOLUME OF TUMOR	LENGTH OF LARVA	LENGTH OF TUMOR	BREADTH OF TUMOR	VOLUME OF TUMOR	LENGTH OF LARVA	LENGTH OF TUMOR	BREADTH OF TUMOR	VOLUME OF TUMOR	
mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	
1.8	0.099	0.050	0.00013	3.0	0.249	0.099	0.00304	3.3	0.199	0.249	0.01482	
2.0	0.099	0.086	0.00083		0.133	0.133			0.083	0.083		
2.2	0.099	0.050	0.00013		0.099	0.099			0.249	0.249		
2.3	0.099	0.086	0.00083	3.0	0.166	0.166	0.00290	3.3	0.199	0.332	0.01147	
2.3	0.249	0.166	0.00356		0.099	0.099			0.249	0.160		
2.3	0.099	0.083	0.00036		0.249	0.249			0.160	0.160		
2.4	0.166	0.249	0.00539	3.0	0.160	0.160	0.00102	3.4	0.332	0.332	0.01905	
2.5	0.249	0.166	0.00356		0.330	0.033			0.332	0.249		
2.7	0.249	0.166	0.00356		0.066	0.066			0.332	0.166		
2.7	0.249	0.166	0.00356	3.0	0.083	0.083	0.00624	3.4	0.160	0.132	0.00145	
2.7	0.332	0.166	0.00539		0.033	0.033			0.332	0.249		
	0.083	0.083			0.033	0.033			0.249	0.249		
	0.083	0.083		0.249	0.249	0.099	0.099					
2.7	0.099	0.166	0.00143	3.0	0.249	0.249	0.01616	3.4	0.249	0.249	0.00808	
2.8	0.099	0.099	0.00080		0.132	0.199			0.332	0.166		
	0.083	0.083			0.249	0.249			0.232	0.266		
2.8	0.249	0.200	0.00521	3.0	0.249	0.249	0.00808	3.4	0.083	0.083	0.00884	
2.8	0.249	0.249	0.00898		0.249	0.166			0.333	0.333		
	0.166	0.099			0.099	0.099			0.083	0.083		
	0.099	0.099			0.332	0.166			0.166	0.033		
2.8	0.083	0.083	0.00395	3.1	0.249	0.166	0.00356	3.4	0.080	0.080	0.00036	
	0.249	0.166			0.249	0.166			0.232	0.232		
	0.033	0.033			0.299	0.166			0.166	0.166		
2.8	0.033	0.033	0.00395	3.2	0.332	0.199	0.00687	3.4	0.265	0.265	0.02356	
	0.033	0.033			0.132	0.083			0.249	0.249		
	0.033	0.033			0.132	0.083			0.050	0.050		
2.8	0.033	0.033	0.00287	3.2	0.248	0.166	0.00463	3.5	0.050	0.050	0.00007	
	0.166	0.166			0.166	0.099			0.332	0.249		
	0.333	0.166			0.083	0.033			0.249	0.249		
2.8	0.199	0.166	0.00273	3.2	0.332	0.249	0.01432	3.5	0.083	0.083	0.00140	
2.9	0.333	0.249	0.01575		0.219	0.166			0.083	0.083		
	0.333	0.166			0.249	0.166			0.249	0.249		
3.0	0.333	0.249	0.01095	3.2	0.249	0.166	0.00612	3.5	0.166	0.332	0.00965	
3.0	0.242	0.166	0.00349		0.083	0.083			0.365	0.249		
3.0	0.333	0.166	0.00480		0.166	0.166			0.083	0.166		
3.0	0.333	0.166	0.00480	3.3	0.265	0.232	0.01455	3.5	0.033	0.033	0.01359	
3.0	0.099	0.099	0.00102		0.166	0.083			0.033	0.083		
	0.099	0.099			0.083	0.083			0.033	0.083		
3.0	0.249	0.166	0.00386		0.083	0.083			0.033	0.083		
	0.083	0.083			0.083	0.083			0.083	0.083		

TABLE 4—*Concluded*

LENGTH OF LARVA	LENGTH OF TUMOR	BREADTH OF TUMOR	VOLUME OF TUMOR	LENGTH OF LARVA	LENGTH OF TUMOR	BREADTH OF TUMOR	VOLUME OF TUMOR	LENGTH OF LARVA	LENGTH OF TUMOR	BREADTH OF TUMOR	VOLUME OF TUMOR
mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
3.5	0.332	0.332	0.01905	4.0	0.166	0.099	0.00085	4.4	0.199	0.083	0.00413
3.5	0.332	0.332	0.01905	4.0	0.249	0.249	0.00808	4.4	0.166	0.199	0.01436
3.5	0.249	0.249	0.01616	4.0	0.332	0.332	0.01905	4.4	0.249	0.332	0.00239
3.5	0.249	0.249	0.01616	4.0	0.166	0.083	0.00590	4.4	0.166	0.166	0.00239
3.6	0.415	0.249	0.01315	4.0	0.166	0.332	0.00955	4.4	0.332	0.332	0.02725
3.6	0.498	0.415	0.04485	4.0	0.332	0.249	0.01076	4.4	0.332	0.083	0.00479
3.7	0.249	0.249	0.00808	4.0	0.166	0.166	0.00239	4.4	0.249	0.083	0.00051
3.7	0.332	0.332	0.01905	4.0	0.249	0.249	0.01346	4.4	0.249	0.249	0.00479
3.7	0.332	0.249	0.01076	4.0	0.166	0.249	0.00197	4.4	0.0332	0.0332	0.00051
3.7	0.664	0.332	0.03832	4.0	0.166	0.166	0.00290	4.4	0.0332	0.0332	0.00051
3.7	0.332	0.242	0.01020	4.0	0.099	0.099	0.00197	4.4	0.0332	0.0332	0.00051
3.7	0.050	0.050	0.00984	4.0	0.099	0.099	0.00197	4.4	0.0332	0.0332	0.00051
3.7	0.160	0.332	0.00984	4.0	0.066	0.066	0.00197	4.4	0.0332	0.0332	0.00051
3.7	0.160	0.080	0.00984	4.1	0.415	0.332	0.02375	4.5	0.332	0.332	0.01905
3.7	0.332	0.249	0.01142	4.1	0.166	0.083	0.00598	4.5	0.249	0.249	0.00838
3.7	0.083	0.083	0.01142	4.1	0.415	0.249	0.01355	4.5	0.083	0.083	0.00479
3.7	0.166	0.066	0.01076	4.1	0.083	0.083	0.01355	4.6	0.332	0.166	0.00051
3.7	0.332	0.249	0.01076	4.1	0.249	0.166	0.00697	4.6	0.099	0.099	0.00479
3.8	0.418	0.166	0.00602	4.3	0.099	0.099	0.00697	4.6	0.332	0.166	0.00479
3.9	0.166	0.166	0.00762	4.3	0.099	0.099	0.00697	4.6	0.332	0.166	0.00479
3.9	0.366	0.166	0.00762	4.3	0.166	0.166	0.00697	4.6	0.332	0.166	0.00479

deviation, 1.154 ± 0.041 . The mean length of the tumor is 0.488 ± 0.011 ; its standard deviation, 0.217 ± 0.008 . The mean breadth of the tumor is 0.362 ± 0.009 ; its standard deviation, 0.174 ± 0.006 . The mean number of tumors is 2.723 ± 0.135 ; its standard deviation, 2.696 ± 0.096 . The mean volume of tumors is 0.008 ± 0.0005 ; its standard deviation, 0.008 ± 0.0004 .

There seems to be no definite relationship between the size of the tumor or the number of tumors and the time of the occurrence of death. The smallest tumor may appear in the largest larva and still cause death. There is, however, a relationship between the occurrence of death and the stage of development of the tumor, as already stated. Death may occur at any period of the larval life, and depends upon a definite stage in the development of the tumor. The lethal factor 7 evidently manifests itself in the form of this lethal tumor.

TABLE 5

CORRELATED VARIATES	CORRELATION	PROBABLE ERROR
Length of larva to number of tumors.....	0.048	±0.065
Length of larva to length of tumors.....	0.127	±0.049
Length of larva to breadth of tumors.....	0.169	±0.048
Length of larva to volume of tumors. . .	0.094	±0.065
Number of tumors to length of tumors..	-0.390	±0.042
Number of tumors to breadth of tumors.....	-0.388	±0.043
Length of tumors to breadth of tumors.....	0.748	±0.022

TABLE 6

Physical constants of distribution

	MEAN	PROBABLE ERROR	STANDARD DEVIATION	PROBABLE ERROR
Length of larva.....	3.742	±0.058	1.154	±0.041
Length of tumor.....	0.488	±0.011	0.217	±0.008
Volume of tumor.....	0.008	±0.0005	0.008	±0.0004
Breadth of tumor.....	0.362	±0.009	0.174	±0.006
Number of tumors.....	2.723	±0.135	2.696	±0.096

THE SEX-LINKED INHERITANCE OF THE TUMOR

The linkage experiments of C. B. Bridges showed that the lethal 7 factor which produces the tumor, and thereby the death of the fly, is sex-linked in inheritance, being located in the X-chromosome (at the locus 0.01).

The X-chromosome, of which the female has two, carries the factor for sex. After the process of maturation in the female, there is one X-chromosome left in each egg, while after a similar process in the male, one half the spermatozoa receive the X-chromosome and the other half its mate, the Y-chromosome. If an X-bearing spermatozoon should fertilize an egg the result will be an XX individual or female. If a Y-bearing spermatozoon should fertilize an egg, the result will be an XY individual, or male. Since the two kinds of spermatozoa appear in equal numbers, males and females are produced in equal numbers. In stocks of flies having lethal factors, however, only half as many males as females appear. Since the recessive lethal factor is present in only one of the two X-chromosomes, one-half of

the males from a lethal bearing mother will get the X-chromosome that carries the lethal factor, and such males will perish. The other half of the males will get the X-chromosome that carries the normal allelomorph of the lethal factor and such males will live. None of the females die, because those that contain the lethal bearing X-chromosome have received also from the father another chromosome with the dominant normal allelomorph of the lethal factor. Therefore since the tumor of lethal 7 has the same inheritance as its lethal factor, the females of that stock do not develop tumors. To produce a female with a tumor, it would be necessary that each parent contain an X-chromosome with a factor for the lethal which is the cause of the tumor; but it is not possible to produce such a female, since all males with tumors die. The tumor is sex-linked in inheritance because the factor for the tumor is contained in, and transmitted by, the X-chromosome. It follows, in fact, the regular order of transmission shown by all sex-linked characters.

POSITION OF TUMOR IN LARVA

Larvae with tumors were selected from cultures A, B, and C. These were etherized just enough to make them quiet while measurements were made of the length of the larvae and of the lengths and breadths of the tumors. The location of the tumors was also noted. They may be found in any segment of the body, as noted in table 7. By plotting the results indicated in table 7, a bimodal curve is obtained (text fig. 2). Though the tumor may occur in any segment, it occurs more often in segments twelve and six.

The figures on plate I are camera lucida drawings of larvae with tumors, showing that the tumor may occur in any segment and that more than one tumor may occur.

REMOVAL OF TUMORS BY OPERATION

Larvae with tumors were washed in sterile water by transferring the larvae from one watch glass with sterile water to another, making the transfer five or six times; then placed upon

moist sterile absorbing paper in a watch glass to be etherized. Care in administering the ether must be exercised, as too much ether will cause the larvae to contract violently, making them

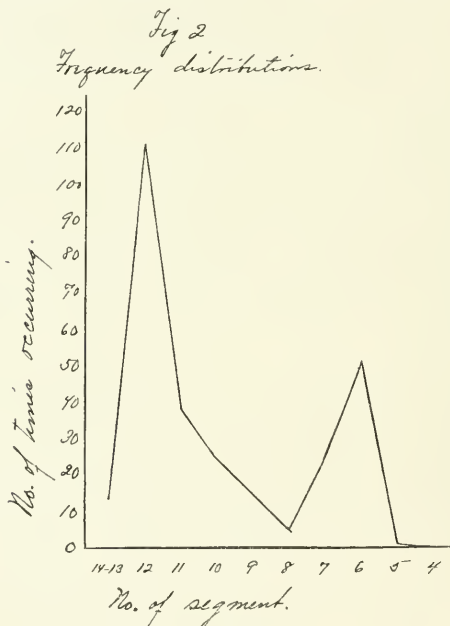
TABLE 7

POSITION OF TUMORS IN SEGMENTS	OCCURRENCES IN CULTURE A	OCCURRENCES IN CULTURE B	OCCURRENCES IN CULTURE C	TOTAL
14-13	5 (5)*	(6)	(2)	(13)
14-13-12	1	2		
14-13-12-11		1		
14-13-12-10		2	1	
14-13-11			1	
14-13-12-11-10		1		
12	11 (22)	26 (55)	20 (34)	(111)
12-11	2	6	4	
12-10	4	1	2	
12-9		6	1	
12-8	1		1	
12-7		6	1	
12-6			2	
12-10-9	3	1		
12-9-7			1	
12-11-10-6			1	
12-7-6			1	
12-11-9		1		
11	9 (11)	5 (15)	4 (12)	(38)
11-9-8		1		
11-8-7			1	
11-7-3			1	
10	4 (12)	4 (9)	(4)	(25)
10-7	1			
9	1 (4)	1 (10)	(2)	(16)
8	1 (3)	(1)	(2)	(6)
8-5	1			
7	4 (5)	5 (11)	4 (9)	(25)
6	12 (12)	16 (16)	10 (13)	(51)
5	(1)			(1)
				(286)

* The numbers in parenthesis indicate the total number of times the tumor has occurred in a given segment.

useless for the operation. When the larvae were quiet and relaxed, they were placed under the binocular. A minute cut was made through the body wall above the tumor with a pair of

iridectomy scissors. Slight pressure was then applied to the body wall to force the tumor out of the incision. The tumor was often forced out by internal pressure as soon as the cut was made. If the cut was the least bit too large, most of the intestine would be forced out also.



TEXT FIG. 2

Larvae with only one tumor were operated upon. The number of tumors removed successfully was about sixteen hundred (1600). About 5 per cent of the larvae from which tumors were removed lived twenty-four hours after the operation. The wound would often be closed within twelve hours. Not any of these larvae pupated. As a control, fourteen hundred nor-

mal larvae were operated upon, a minute cut being made through the body wall. Of these, about 5 per cent recovered from the operation; these afterward pupated and emerged as normal flies. Since only a small percentage of these controls survived the operation, it is possible that the death of the majority of the larvae from which the tumors were removed might likewise have been due to the shock of the operation, but on the other hand, the small percentage (the same as that of the control, however) that survived the operation and lived for fifteen hours and more may have died from the injurious effects that had already been produced by the tumor. That the operation was of beneficial effect upon the larvae having tumors can scarcely be doubted in view of the fact that unoperated larvae seldom survive so long as the operated, which in as large numbers as 5 per cent successfully passed the first stage of recovery, though they ultimately succumbed.

Tumors were also removed, about fourteen hundred in number, and inserted into as many normal larvae. As a control, sterile charcoal was inserted into normal larvae. A cut was made through the body wall of the normal larvae, and the tumor (or charcoal) was then pushed into the body cavity with a small arrowshaped blade. Insertion of the piece of charcoal was as difficult as that of the tumor, if not more difficult, because the charcoal was more easily forced out again by internal pressure; thus the procedure often had to be repeated several times, and the piece forced further into the body cavity. The tumor, on the other hand, moist with blood, would adhere more readily. Nearly 5 per cent of the larvae into which fragments of charcoal were inserted survived the operation, pupated, and emerged as normal flies; in many of these, particles of charcoal were present in the abdominal cavity of the fly, while in other cases they were left behind in the pupa case. The death of most of the larvae, 95 per cent, into which tumors were inserted, was in all probability due to the shock of the operation, as shown by the controls, while the death before pupation of the remaining 5 per cent must have been due to the inserted material.

Tumors were ground in Locke's solution with all necessary aseptic precautions, and a small portion of the mixture was forced into the body cavity of normal larvae with a capillary pipette. Out of one hundred larvae thus operated upon, forty survived the operation; i.e., the wound was healed and the larvae were active and feeding two hours after the operation. Although no visible tumor developed, none of these pupated, death occurring within twelve hours. As a control to this experiment, an equal number of larvae were pierced with capillary pipettes; into half of these was forced a small amount of Locke's solution. Fifty per cent of those pierced with the pipette, without any injection, survived the operation, pupated, and emerged as normal flies. Of those into which was forced the Locke's solution, nearly 40 per cent survived, pupated, and became flies. It is evident that the tumor cell suspension produces a toxic effect upon the larvae.

THE INFLUENCE OF x -RAYS UPON THE TUMOR

The following experiments were performed in coöperation with Dr. J. L. Kantor, who offered the use of his apparatus for this purpose. The current used was generated by an interrupterless transformer. The larvae and pupae to be exposed were placed upon a glass plate 30 cm. from the anode. The rays were filtered through 1 mm. aluminum. The amount of current used in each of the following experiments was 6 milliamperes, the Coolidge tube "backing up" to a 4-inch spark gap.

Larvae with tumors, when exposed every day for eight fifteen-second periods interrupted by five-second periods of rest, lived from six to fifteen hours longer than larvae of the same length not exposed to x -rays, as shown in table 8.

The normal larvae exposed to the same dose of x -rays showed a tendency to pupate earlier than those not exposed. This early pupation was not due to drying, as the absorbing paper upon which the larvae rested was kept moist.

All the eggs, larvae, and pupae from five pairs of flies were exposed to x -rays every day until the new generation emerged from the pupae. The results, shown in table 9, do not indicate

any effect upon the development of the tumor as the ratio of twice as many females as males remains the same, the larvae with tumors dying as before. The only difference noted was the

TABLE 8

LARVAE WITH TUMORS	NORMAL LARVAE	DURATION OF EACH EXPOSURE	NUMBER OF DAYS EXPOSED	DATE OF FIRST EXPOSURE	TIME OF DEATH OF LARVAE WITH TUMORS	PUPATION OF NORMAL LARVAE
20	20	Eight 5-second periods interrupted by 5-second periods of rest	4	July 12, 1916	2 July 25 7 July 26 3 July 27 8 July 28	5 July 25 15 July 26
10	10	Eight 5-second periods interrupted by 5-second periods of rest	3	July 25, 1916	3 July 27 7 July 28	4 July 26 6 July 27
10	10	Eight 5-second periods interrupted by 5-second periods of rest	3	July 27, 1916	5 July 29 5 July 30	3 July 28 7 July 29
10	10	Eight 5-second periods interrupted by 5-second periods of rest	3	July 28, 1916	3 July 29 7 July 30	4 July 30 6 July 31
10	<i>Control</i> 10	0	0	<i>Date picked</i> July 25, 1916	3 July 25 7 July 26	4 July 27 6 July 28

emergence of the flies exposed to x -rays twenty-four to thirty-six hours earlier than in the control experiments.

The eggs, larvae, and pupae of four pairs of flies from pair II of table 9 were also exposed to x -rays every day. A similar treatment was given to all the offspring of two pairs of flies from II.3 (table 10). Three generations of flies were thus exposed

daily to *x*-rays from the egg stage of the first generation to the adult stage of the third generation. The results in table 9 indicate no effect upon the larvae with tumors.

TABLE 9
First generation

NUMBER OF PAIR	DURATION OF EXPOSURE	NUMBER OF DAYS EXPOSED	DATE OF PAIRING AND FIRST EXPOSURE	DATE OF APPEARANCE OF OFFSPRING	TOTAL OUTPUT	
					R [♀] SW	R [♂] SW
I	Eight 15-second periods interrupted by 5-second periods of rest	22	July 27, 1916	August 6, 1916	72-66	1-69
II	Eight 15-second periods interrupted by 5-second periods of rest	22	July 27, 1916	August 6, 1916	55-37	0-49
III	Eight 15-second periods interrupted by 5-second periods of rest	21	July 28, 1916	August 7, 1916	45-36	0-47
IV	Eight 15-second periods interrupted by 5-second periods of rest	21	July 28, 1916	August 7, 1916	63-65	2-57
V	Eight 15-second periods interrupted by 5-second periods of rest	21	July 29, 1916	August 8, 1916	54-42	2-49
<i>Control</i>			<i>Paired</i>			
I	0	0	July 27, 1916	August 9, 1916	59-44	0-35
II	0	0	July 28, 1916	August 11, 1916	57-40	1-36

Larvae operated upon were also exposed to *x*-rays. The results in table 11 show that larvae with tumors removed, when exposed to *x*-rays, live from six to twelve hours longer than the controls or larvae with tumors removed but not exposed to *x*-rays. Similar results are obtained when normal larvae into

which tumors have been inserted are exposed to x-rays, as indicated in table 12.

TABLE 10
Second and third generation

NUMBER OF PAIR	DURATION OF EXPOSURE	NUMBER OF DAYS EXPOSED	DATE OF PAIRING AND FIRST EXPOSURE	DATE OF APPEARANCE OF OFFSPRING	TOTAL OUTPUT	
					R ♀ ⁸ W	R ♂ ⁸ W
II 1	Eight 15-second periods interrupted by 5-second periods of rest	25	August 11, 1916	August 19, 1916	42-44	1-48
II 2	Eight 15-second periods interrupted by 5-second periods of rest	25	August 11, 1916	August 21, 1916	45-41	0-46
II 3	Eight 15-second periods interrupted by 5-second periods of rest	25	August 11, 1916	August 20, 1916	54-59	0-53
II 4	Eight 15-second periods interrupted by 5-second periods of rest	25	August 11, 1916	August 20, 1916	57-48	1-51
II 3-1	Eight 15-second periods interrupted by 5-second periods of rest	16	August 20, 1916	August 29, 1916	48-54	1-37
II 3-2	Eight 15-second periods interrupted by 5-second periods of rest	16	August 20, 1916	August 28, 1916	37-34	0-27
<i>Control</i>	0	0	<i>Paired</i> August 11, 1916	August 22, 1916	43-45	0-39

Of the normal larvae operated upon as a control to the experiment in which tumors were removed and inserted, one hundred were exposed to x-rays. Table 13 shows a slight increase in the

TABLE 11

LARVAE WITH TUMORS REMOVED	DURATION OF EXPOSURE	DATE OF OPERATION AND FIRST EXPOSURE	NUMBER OF DAYS EXPOSED	HEALING OF WOUND	OCCURRENCE OF DEATH
	<i>minutes</i>				
10	2	August 28, 1916, 4.30 p.m.	2	Before 12 hours	2 before 24 hours; 8 after
10	0	Operation August 28, 1916	0	After 12 hours	All before 24 hours
10	2	August 29, 1916, 4.30 p.m.	2	Before 10 hours	3 before 24 hours; 7 after
10	0	Operation August 29, 1916	0	After 12 hours	All before 24 hours
10	2	August 30, 1916, 4.30 p.m.	2	Before 10 hours	4 before 24 hours; 6 after
10	0	Operation August 30, 1916	0	After 12 hours	All before 24 hours
10	2	August 31, 1916, 4.30 p.m.	2	Before 10 hours	2 before 24 hours; 8 after
10	0	Operation August 31, 1916	0	After 12 hours	All before 24 hours
10	2	September 5, 1916, 4.30 p.m.	2	Before 10 hours	3 before 24 hours; 7 after
10	0	Operation September 5, 1916	0	After 12 hours	All before 24 hours

TABLE 12

LARVAE WITH TUMORS INSERTED	DURATION OF EXPOSURE	DATE OF OPERATION AND FIRST EXPOSURE	NUMBER OF DAYS EXPOSED	HEALING OF WOUND	OCCURRENCE OF DEATH
	<i>minutes</i>				
10	2	August 29, 1916, 4.30 p.m.	2	Before 10 hours	3 before 24 hours; 7 after
10	0	Operation August 29, 1916	0	After 12 hours	All before 24 hours
10	2	August 30, 1916, 4.30 p.m.	2	Before 10 hours	4 before 24 hours; 6 after
10	0	Operation August 30, 1916	0	After 12 hours	All before 24 hours
10	2	September 6, 1916, 4.30 p.m.	2	Before 10 hours	2 before 24 hours; 8 after
10	0	Operation September 6, 1916	0	After 12 hours	All before 24 hours
10	2	September 7, 1916, 4.30 p.m.	2	Before 10 hours	3 before 24 hours; 7 after
10	0	Operation September 7, 1916	0	After 12 hours	All before 24 hours
10	2	September 8, 1916, 4.30 p.m.	2	Before 10 hours	5 before 24 hours; 5 after
10	0	Operation September 8, 1916	0	After 12 hours	All before 24 hours

TABLE 13

NORMAL LARVAE	DURATION OF EXPOSURE <i>minutes</i>	DATE OF OPERATION AND FIRST EXPOSURE	NUMBER OF DAYS EXPOSED	HEALING OF WOUND	NUMBER OF FLIES THAT EMERGED
20	2	August 30, 1916, 4.30 p.m.	2	Before 10 hours	1
20	0	Operation August 30, 1916	0	After 10 hours	0
20	2	September 5, 1916, 4.30 p.m.	2	Before 10 hours	0
20	0	Operation September 5, 1916	0	After 10 hours	1
20	2	September 6, 1916, 4.30 p.m.	2	Before 10 hours	2
20	0	Operation September 6, 1916	0	After 10 hours	1
20	2	September 7, 1916, 4.30 p.m.	2	Before 10 hours	2
20	0	Operation September 7, 1916	0	After 10 hours	1
20	2	September 8, 1916, 4.30 p.m.	2	Before 10 hours	1
20	0	Operation September 8, 1916	0	After 10 hours	1

percentage of the number of larvae surviving the operation, as compared with that of the number of normal larvae operated upon but not exposed to x-rays.

In the x-ray experiments, there is no indication that the x-rays had any obvious effect upon the tumor. It developed and remained as lethal as ever in all the flies exposed, even when the exposure was repeated each day continuously through the entire life cycle of three generations of flies. More definite conclusions cannot be drawn, however, until the experiment has been repeated using varying doses of the x-rays. It may be that the rays, in the dose used, were not of a strength appropriate to affect the tumors.

There was a decided influence upon the rate of healing of the wound. It is possible that the rays had a tendency to decrease infection, thus increasing the rate of healing and prolonging the life of the larvae.

DEVELOPMENT OF THE TUMOR

The tumor may take its origin in various regions of the body of the larva. It may develop in the ganglion of the proventriculus, located between the brain and the proventriculus. When the tumor develops in this region, the salivary glands usually become affected since they are innervated by this ganglion (see fig. 5, plate I).

The tumor may develop in groups of embryonic cells or imaginal rudiments located in the dorsal region of the thoracic segments and near the posterior end of the abdomen. The tumor shown in text figure 1 took its origin in an aggregation of embryonic cells near the posterior end.

|| In the early stages of the development of the tumor there is a deposit of pigment, followed by a rapid proliferation of the cells which, as development goes on, push the older cells filled with pigment toward the periphery and there form the laminated layers of pigment with the flattened peripheral cells shown in text figure 1.

SUMMARY

1. A sex-linked factor in a stock of *Drosophila* called lethal 7 manifests itself in the form of a lethal tumor in the sense defined on page 282.

2. The time at which death takes place depends upon the stage of development of the tumor.

3. Five per cent of the sixteen hundred larvae from which tumors were removed survived the operation and lived longer than they would have done without the operation. All of these died before pupation, death being probably due to injurious effects already induced by the tumor before its removal.

4. Four per cent of fourteen hundred normal larvae into which tumors were inserted survived the operation, but died before pupation. Forty per cent of one hundred normal larvae into which tumor cell suspension was injected survived the operation but died later. The occurrence of death before pupation was probably due to the injected material.

5. There is no obvious effect upon the tumor when larvae containing it are exposed to *x*-rays.

6. The tumor may develop in the proventriculus ganglion, or in the salivary glands, or in the imaginal rudiments of the thorax and abdomen.

7. The tumor is epithelial in structure, and pigment producing.

In conclusion, I wish to acknowledge my indebtedness to Prof. T. H. Morgan for his interest in the progress of my work and for his many valuable suggestions, as well as to Dr. C. B. Bridges for the lethal 7 stock which has enabled me to do this work.

PLATE I

EXPLANATION OF FIGURES

All the drawings were made with camera lucida, 16 mm. objective and $6\times$ ocular.

Fig. 1. Dorsal view of the anterior (above) and posterior (below) portions of a larva with sixteen tumors.

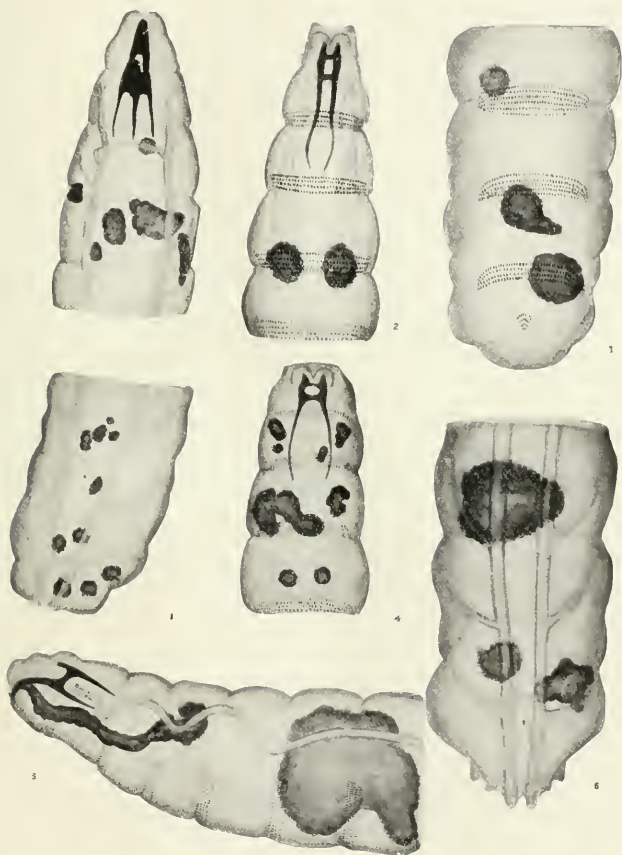
Fig. 2. Ventral view of the anterior end of a larva with a tumor at the end of each salivary gland.

Fig. 3. Ventral view of the posterior end of a larva with three tumors.

Fig. 4. Ventral view of the anterior portion of a larva with tumors in the proventricular ganglion and the salivary glands.

Fig. 5. Lateral view of a larva with one large tumor in segments 8-9 and the left salivary gland entirely impregnated with pigment.

Fig. 6. Dorsal view of the last three segments of a larva with three tumors.



THE BIOLOGICAL TREATMENT OF CANCER¹

G. FICHERA

University of Cagliari

Received for publication, September 27, 1917

ANTIPARASITIC METHODS

It is the purpose of this paper to review critically some of the methods and results of the past two decades in cancer research, a branch of pathology which has not failed to share in the general progress of medicine. During this period innumerable experiments have been performed, and the most diverse materials employed, in an endeavor to solve the problem of the cure of cancer.

Thus Bra has employed cultures of *Nectria ditissima*, a parasitic myxomycete occurring in certain plants, and causing a disease resembling cancer in man. Cultures heated to 120° and filtered cause a febrile reaction in cancerous patients which is not found in healthy persons.

Mongour and Gentes, employing this method, found that while the pain, the hemorrhage, and the discharge ceased, cure did not take place, and a similar result has been recorded by Parlavecchio.

Bra later prepared an anticancerous vaccine from a parasite similar to that of plants, but obtained from human tumors. This vaccine, according to Silva and Vidal, caused a febrile reaction but no cures.

Schmidt stated that *Mucor racemosus* is the causative agent of cancer, and that preparations of its cultures exert a therapeutic effect in cancer patients. He published the statement that he

¹ The Editor wishes to thank Dr. Karl M. Vogel for his kindness in translating this article from the Italian.

The author has not read the proof.

had succeeded in increasing its virulence in rats to such a point that in successive inoculations up to the eighth generation he finally achieved 100 per cent of results in 8 rats, although in the first 40 animals tumors had developed in but 2 cases. Furthermore, treatment with dead cultures from tumors obtained in this way gave 20 to 25 per cent of cures.

In view of the negative results of Schuberg, Baisch, and von Dungern and Werner, these experiments may be regarded as quite without value.

Nevertheless, Schmidt and others have reported clinical cases of neoplasm cured by means of cancerin or antimeristem; thus Claes recorded a recurring sarcoma of the maxilla, Neander a neoplastic stenosis of the rectum, Aronsohn a cancer of the larynx, and Jenssen a canceroid of the tongue. But a study of these cases gives rise to some doubt regarding their nature. For example, Aronsohn's patient was a syphilitic with multiple laryngeal nodules, in whom the diagnostic reaction of Schmidt was negative. In Jenssen's case, again, the patient had an ulcer of the tongue in which the *Treponema pallidum* was easily demonstrable; cure resulted only after six months and a half of treatment, and was followed by abscess formation and subsequent scarring (gumma?).

Furthermore, Schmidt's conclusions have been severely criticized by Dreesmann on the basis of observations which he considered of positive value, and by Shaw-Mackenzie. Glenton Myler, also, in 9 cases seen in the Middlesex Hospital, noted the absence of any diagnostic reaction and of any therapeutic effect.

Beresnegowsky, in 7 cases of inoperable carcinoma at the Heidelberg Clinic, had nothing but negative results, while Winkler observed no effect on the tumors and called attention to the serious disturbances produced by the injections.

Beresnegowsky reported 2 patients who died without having been benefited and were examined histologically at biopsy and autopsy under Orth's direction. From these cases, one a recurrent gelatinous carcinoma of the breast, the other an infiltrating cancer of the larynx and epiglottis, Beresnegowsky concluded that neither from the clinical history, the autopsy, nor the mi-

eroscopic examination, could any influence on the tumor be admitted, for the growth was not modified, metastases were not prevented, and the structure was not altered; the only definite result, he added, was the production in the first case of large multiple subcutaneous abscesses.

Czerny, wishing to reinforce the action of radium, tried out the combined action of serum and toxin. For this purpose the cancroin of Schmidt was tested on 45 patients, 14 of whom carried out the complete treatment, while others continued it only for a time or refused to go on with it because the first injection caused inflammation and fever. Czerny pointed out that in cachectic patients with visceral metastases in important organs the treatment should not be attempted, and concluded that although in some cases there might have been seen a temporary modification of the disease, cure was not brought about in any. Similar reports have been made lately by Werner, Sick, Kolb, Bagge, Nordt, and Stockmann; in no case of genuine neoplasia has antimeristem shown any elective action worthy of note.

Wlaeff, an adherent of the theory of the blastomycetic origin of tumors, prepared a specific serum and immunized animals against blastomycetes obtained by culture from human tumors; and having noted that the serum protected rats and monkeys from blastomycetic infection, he tried it in human beings. Serum obtained from pigeons, chickens, geese, or donkeys and administered subcutaneously every five to eight days in doses of from 7 to 12 cm. to patients without metastases, was said to produce an improvement in the general condition, a reduction in the volume of the tumor, and an arrest of its development. Permanent cure did not take place, however, the neoplasms later resuming their growth.

Biondi, in a discussion of the serotherapy of tumors presented before the Italian Surgical Society in 1895, stated that the serum of animals immunized with the blastomycete of Sanfelice was without any influence on either sarcomas or epitheliomas, while Lucas-Championniere, Le Dentu, and Berger obtained only transitory arrest with Wlaeff's serum.

The significance of blastomycetes as neoplastic agents has been denied also, and on the basis of accurate and extensive research, by Laederich and Duval, Franchetti, Stropeni, Cao, Tiberti, Donati, Alessandri and Zapelloni, Magnini, and finally by Galeotti, who was formerly inclined to ascribe etiological importance to them.

In regard to the therapeutic action of the serum prepared by Sanfelice and used by him in cases of lymphosarcoma and carcinoma of dogs, it must be objected that spontaneous regression of this type of lymphosarcoma has been reported by Sticker, Bashford, Beebe and Ewing, and Veratti, and that many observers are not willing to include the growth among the true tumors.

In three cases of cancer of the breast in dogs, which were treated with the serum, disappearance of the growth did not take place, but Sanfelice reported degenerative changes and a connective tissue reaction; this, however, Veratti and Vidal considered evidence of partial spontaneous regression.

Doyen has given the name *Micrococcus neoformans* to a parasite which he believes to be the cause of tumors. Intraperitoneal inoculation in white mice is said to have caused the formation of tumors about the liver and pancreas, and an inflammatory reaction of the lung with hyperplasia of the bronchial epithelium. And foci of melanosa were said to have followed the intraperitoneal injection of an emulsion of a human pigmented tumor mixed with *Micrococcus neoformans*. In rats, inflammatory changes were observed in the large intestine, with changes in the glands, and Doyen described also a papillary carcinoma of the bladder, a cystic fibroma of the tube, epithelial growths of the lung accompanied by myxocartilaginous new growths, and several lipomas.

But the variety of type assumed by these growths, no less than the lack of precision with which they are described, suggests doubt as to the actual nature of the lesions encountered, and the suspicion is aroused that they were either chance findings or simple inflammatory reactions. The example considered by Doyen the most valuable only increases these doubts, for the

"melanotic sarcoma" above mentioned may be explained by mere phagocytic pigmentation of the reactive tissue at the site of the injection.

Doyen administered preparations of his bacillus for therapeutic purposes, also, but as the cases were treated at the same time by surgery, x-rays, radium, thermocoagulation, and heat otherwise applied, there was such an accumulation of therapeutic procedures that it is difficult to determine what part of the result can be attributed to the antineoplastic vaccination. The diagnoses were not confirmed microscopically, and even clinically were far from certain. Histological reports are generally absent, even in the cases that were subjected to operation, though they would have been of great interest in establishing the diagnosis and determining the changes referable to the treatment.

Furthermore, Hornus, in his inaugural dissertation on this subject, prepared under the guidance of Doyen, speaks only of delayed development, amelioration of the symptoms, improvement in the general conditions, and prolongation of life, producing no evidence that might lead one to accept the treatment as a cure. Nor did Berger, Monod, Nelaton, Kirmisson, and Delbet observe any improvement in 26 patients treated by Doyen, and followed for five months; unfavorable observations have been reported also by Vidal and Bender, Paine and Morgan, Pattison, Thomson, Musgrave, Woodman, van Ermengem, and Debaisieux.

Nobody, in short, has yet succeeded in obtaining reliable results from methods of treatment based upon a specific causative agent. These results, furthermore, have been no better than those obtained on the basis of a parasitic etiology acknowledged to be erroneous, as, for example, in the case of Adamkiewicz's hypothesis or of procedures based upon germs to which no pathogenic importance is attached, like the streptococcus of Fehleisen, or the *Bacillus prodigiosus* in the methods of Coley and Emmerich and Scholl.

PASSIVE IMMUNIZATION WITH TUMORS

Another group of investigators, without trying to determine the identity of the specific agent concerned in neoplasia, has attempted to secure active or passive immunization with tumor, on the hypothesis that some virus is contained in its tissues or that the tumor cells themselves are a virus.

This group includes Richet and Hericourt, who in 1895 immunized animals with osteosarcoma; the tumor was finely triturated and filtered through gauze and the liquid so obtained was injected intravenously into donkeys, dogs, or horses. The serum of these animals, collected seven to twelve days later, was introduced subcutaneously in doses of 3 cc., either in the neighborhood of the tumor or at a distance from it. It was asserted that noteworthy improvement in the general condition set in, that the tumors diminished in size, hemorrhages were arrested, ulcerated surfaces became clean, and that partial cicatrization resulted, though without permanent benefit being obtained. After a very short period the tumors again began to grow and the general unfavorable condition progressed in spite of continuation of the treatment. This was further accompanied, moreover, by evidences of intolerance, among which vomiting, diarrhea, lumbar pain, skin eruptions, headache, and fever were notable. Similar findings have been reported by Boureau, Boinet, Brunner, Bosc, Ceci, Barlerin, Biondi, Pascale, De Gaetano, Salvati, and Parascandolo.

Various modifications of the serum treatment have been suggested, especially since the early researches of Metalnikoff, Ehrlich, and von Dungern on cytotoxins. These suggested a specificity of cytolytins, and gave rise to the hope that a cytolytin might be prepared which would attack only tumor cells. The attempts begun by Richet and Hericourt were therefore continued by other investigators, among whom were Poncet and Dor, Charcot, Jensen, von Leyden and Blumenthal, Hoyten, Borrel, Lomer, Loeffler, Vidal, etc.

Dor, in 1900, injected a goat with a melanotic tumor and introduced the serum so obtained into two persons suffering

from melanosarcoma. After a period of subjective and objective improvement the first patient was lost sight of, while in the second case the treatment was interrupted by lack of serum.

Leyden and Blumenthal treated goats and sheep with epithelium from the breast, liver, or uterus, and injected the serum into persons suffering from epithelial new growths of the corresponding organs, but except for temporary improvement and transitory changes in the consistency and volume of the primary tumor and its metastases, no action was discernible.

Hoyten injected fluids from human carcinoma into dogs and inoculated their serum into patients; the results were more evident in the metastases than in the original tumor.

Lomer reported a case of recurrence in the scar of an operation for carcinoma of the breast. Half of this nodule was used for histological diagnosis, and the remainder disappeared following a series of injections of animal serum obtained after treatment with human epithelium. In the same way an axillary metastasis that appeared later was caused to regress.

Vidal has recently affirmed that it is possible to obtain a serum of considerable efficacy by injecting animals with human tumor of the same type as that which is to be treated. For this purpose he carried out the method of Besredka for antibacterial serums, injecting a fresh emulsion of tumor cells sensitized with the corresponding serum previously obtained. Furthermore, during the treatment of the animal from which the serum was to be obtained, he injected substances capable of bringing about a hyperleucocytosis in order to increase the antibody content. Curative sera thus prepared, which should possess specific properties and particularly that of cytotoxicity for the tumor cells, require a long period of administration because their action is slow. During this time, according to Vidal, the body of the patient reacts by the production of neutralizing substances forming an immune antiserum. To avoid this drawback, he prepared another serum from dogs treated with neutralizing blood from patients who for some time had been subjected to the immune serum, or with pleural or peritoneal effusion fluid from the same patients; in this way he obtained an anti-immune serum, or

serum N. After prolonged injections of an immune serum and of this serum N, Vidal obtained in some patients complete regression of their tumors.

But in spite of the various modifications suggested by Richet and Hericourt, serotherapy has not fulfilled expectations, and has even lost a great part of its experimental basis. Thus Jensen, who was among the first to carry out attempts at specific serotherapy in animals, has acknowledged that the regression observed by him in the transplanted tumors of mice that had been injected with serum from immunized rabbits, was spontaneous; that is, it often occurs without the use of any specific serum. This agrees with the experience of Bashford, Murray, and Cramer, who did not observe any effect from serotherapy when treated mice were compared with untreated controls.

Loeffler, who attempted to obtain an active serum from immunized donkeys, was obliged to admit that such a serum precipitated normal elements in the same degree as tumor cells.

In addition to a lack of specificity in the serum of immunized animals, and to its inefficacy, it is to be noted that the partial and temporary results referred to immune sera have also been described after the use of normal serum (Arloing and Courmont, and Augagneur).

ACTIVE IMMUNIZATION WITH TUMORS

Beside passive immunization, active immunization by vaccination with tumor has been attempted, in the hope of obtaining specific antibodies in the patient himself. Thus, Hoyten, in 1899, injected into the neoplasm tumor juices diluted with physiological salt solution, and reported favorable results.

von Leyden and Blumenthal spoke favorably of active immunization carried out according to their hypothesis. In the first notes published, it was stated that the expressed juices of new growths obtained from human beings at operation were tried on three patients. The tumors themselves did not undergo any important change, but indurated lymph-nodes in the neighborhood grew smaller and improvement was noted in the pain and

in the general condition; the nature of the lymphadenoid enlargement, however, was somewhat doubtful, since the neoplasms were ulcerated and therefore infected. Two of the cases are reported to have died.

Müller, trusting in the bactericidal action of glycerin, prepared his material by placing fresh tumor in 80 per cent glycerin; the mixture was kept for forty-eight hours at 37° and then diluted with physiological salt solution and injected in the mammary region. Seven cases were treated, six of which had carcinoma of the uterus and one an ovarian cyst undergoing carcinomatous change. The injections frequently produced abscesses and febrile symptoms, but no cure.

Other attempts at active immunization with tumor have been made without decisive results by Caan, Rovsing, Odier, Delbet, Graff, and Ranzi. Delbet, for example, injected into patients after radical operation the entire tumor reduced to a pulp; this material, without the addition of any bactericidal substance, was suspended in a small amount of physiological salt solution and introduced subcutaneously in two, three, or four different regions, according to its volume. It must be mentioned, however, that he preceded the operation and injection by a course of radiotherapy and the copious painting of raw surfaces with tincture of iodine, and followed it by the application of at least 3 cgm. of radium bromide between the sutured surfaces. This was allowed to remain in position for twenty-four hours, and ten days later *x-ray* treatment was again instituted. Only a short time was necessary to show that the method produced implanted tumors, a consequence which supervened in the cases of von Graff and Ranzi also.

Coca and Gilman have also taken up the matter of active immunization, inspired by the researches of Metalnikoff, von Dungern, and Hirschfeld. The patient's tumor was reduced to small fragments with scissors immediately after removal, repeatedly subjected to pressure, suspended in physiological salt solution, centrifuged, decanted, and finally treated with 0.5 per cent of phenol or toluol. The injections were generally carried out about six hours after the addition of the phenol, though

sometimes earlier when the extract was uncontaminated. The dose of the tissue for each treatment varied from 10 to 25 grams, the fluid being introduced into the abdominal wall through four to six different punctures. The inoculation often gave rise to a local reaction, but in no case did sufficient time elapse to enable one to come to a decision regarding the value of the method; in most cases there was a period of only a few days or months between the operation and the report. Furthermore, microscopical examinations and clinical reports are lacking.

The dangers inherent in active immunization when it is carried out by methods above mentioned have been clearly indicated by Wolff and Blumenthal. Wolff makes the following comment: "Autovaccination, as we have so far come to know it, always involves the danger that living cancer cells (or if one upholds the parasitic theory as von Leyden and others do, living parasites) may be introduced, to give rise later to the formation of new tumors."

HISTOGENETIC CHEMOTHERAPY

While stress had been laid on the principles of active immunization as recently as 1910 with the technic and the results just recounted, I had undertaken comparative observations on embryonal and tumor implantation as well as on the action of histogenic autolysates some years previously, and had treated tumors in animals and in man with fetal tissue or with tumor cells. My research and that of my collaborators was founded on the hypothesis of oncogenic disequilibrium, which would refer the origin of tumors to a state of disharmony, a disturbance of the factors regulating proliferation, during which they pass from under the control of the enzymes, hormones, and histolytic products which ordinarily guide them.

Histotherapy on a biochemical basis, or *histogenetic chemotherapy*, attempts to cure oncogenic disturbances and their effects by stimulating oncolysis. It is naturally limited by its own nature, as it is not possible to suppress all the abnormal functional activities to which the origin of tumors is due, nor is it possible to exceed the limited dose that can be injected.

The earliest experiments were carried out with autolysates and filtrates; the clinical course of transplanted tumors in relation to the host, spontaneous involution, and acquired resistance were also investigated, and the conclusion was finally reached that resistance depends on the absorption of histogenic substances and on the reaction produced by these. Later I undertook an investigation of the regression provoked by histogenic substances, either embryonal or neoplastic, and was able to establish a parallel between autolysis *in vivo*, by immunization, and autolysis *in vitro* by the method of histogenic therapeutics. The treatment was applied also to 36 patients with inoperable malignant growths, with a result which was summed up in 1910 as follows:

Objectively there may be an arrest or even a disappearance of the tumor, and anatomical changes may be observed which are sometimes definite enough to permit the assumption of local cure at least. Thus in favorable cases there may be observed a cytolysis of the tumor cells, gradually increasing until these have entirely disappeared; an intense small cell infiltration which is more pronounced about the blood-vessels; the appearance of numerous microphages, macrophages, and plasma cells; active connective tissue and vascular infiltration invading all portions, together with some giant cells; replacement by dense connective tissue; in short, the cessation of all phenomena associated with infiltration and growth. The tumor becomes soft and fluctuating, and aspiration in these cases reveals a clear fluid containing whitish flakes in suspension and having the character of a transudate. All these changes are perfectly comparable to those which I have already described in rat sarcomas treated by means of autolysates, and may be compared, also, to those characteristic of sarcomas or carcinomas which have been transplanted into rats immunized with embryonal tissues. They are similar, too, to those seen in receding tumors and in grafts of embryonal tissue in the second period of their cycle; that is, in the phase of regression and disappearance. It is a question, therefore, of physiopathological phenomena which can be explained according to general laws and which go on with greater intensity in accordance with the combined action of various factors.

The value of histogenic chemotherapy was promptly confirmed by numerous other investigators, and autolysates or

extracts have since then been generally used; by some authors in the way just described, by others through vaccination, which, under these conditions, does not involve the risk of implanted tumors which is present when emulsions are used.

For example, Abderhalden and Kohlhardt have referred explicitly to these researches in their experimental and clinical observations. They attempted to utilize only the antiblastic reaction obtained according to the technic and principles of protective ferments, which they were among the first to study intensively and which have been accepted by many others, at least so far as the specificity of tissues and lytic ferments is concerned.

Blumenthal desired to see the discussion limited to neoplastic tissue, since he denies that the same therapeutic or immunizing results can be obtained with normal tissue as with homologous tumor. But to deny the presence of resistance after treatment with normal tissue is to do away with the researches of Bashford, Schöne, Michaelis, Borrel and Bridé, Lewin, Apolant, Moreschi, Da Fano, Levin, Brancati, Magnini, Biach and Weltmann, Higuchi, Woglom, Loeb and Fleisher, Krongold, Jowleff, and Roffo; yet these have definitely proved the existence of immunity following treatment with skin, embryo emulsion, blood, mammary gland, liver, spleen, and thyroid. To deny this refractory state is, in fact, to deny some of the most important findings in the entire field of cancer research, and, leaving aside the various ideas concerning specificity, would mean abolishing the basis on which vaccination is founded, firmly established as this is by the tuberculin reaction and the resistance to transplanted tumors caused by absorption of injected tissues.

Recent observations, carried out especially in Italy, suggest an easy explanation of the curative mechanism of tissues in general. These have included the investigation of cancerous organs; of inoculation of tumor grafts into various organs; of the immunizing and therapeutic power of extracts from normal and pathological organs; of the effect upon receptivity or resistance of the removal of different organs; of differences between

normal and immune animals; and of the oncolytic variations in the serum of normal or tumor-bearing animals. Among the Italian investigators who have worked on these subjects are the following; Fichera, Ricci, Perez, Gussio, Almagia, Brancati, Magnini, Cimatori, Caravani, Mazza, Piantoni, Donati D'Agata, Serafini; the list of those in other countries includes von Graff, Apolant, Korentschwesky, Sweet, Saxon, Hilario, Loeb, and Freund and Kaminer.

The results of their observations lead to the conclusion that certain organs possessing active oncolytic and hyperplastic properties in normal or immune animals, are inactive in advanced life or in the presence of a large tumor; and furthermore, that the histogenic substances which have been found efficacious act both directly and indirectly.

The *direct* method depends on cytolytic ferments contained in the various tissues and organs (the isoferments of neoplastic autolyzed tissues, and the heteroferments of normal tissues or those undergoing active histolysis). The *indirect* is represented by stimulation of the organs producing oncolytic substances; under favorable conditions this can be effected by the constituents of various tissues.

These observations and conceptions are important, enabling one to understand, for example, the varying efficacy of the different tissues in proportion to their original content of oncolytic substances and the different degrees of antitumorigenic reaction which they can produce.

The observation by Bertrand, Daels and Deleuze, Sellei, Lunckenbein, and Schubert, that unsuitable dosage may cause an increase in the growth of a tumor instead of regression, was demonstrated some time since by me; and Gussio, Brancati, and Caravani hold similar views. This unfavorable action of large doses explains the negative results obtained in man by Austoni and Carraro, while, in considering the work of Uffreduzzi and Morpurgo on rats and mice, one must remember that chloroform rapidly inhibits the therapeutic action of autolysates, as has been shown by Levin, Lewin, Bauer, Latzel, and Wessely. Again, degenerative changes may be followed by

extension of the growth, with liberation of autolytic products and the institution of a negative phase marked by increasing failure of the oncolytic system and a hastening of the clinical course. The same condition follows intense radiation or the administration of large amounts of colloidal substances; these, instead of stimulating, seriously injure the inhibitory organs and bring about those unfavorable effects described by some writers. There is a connection between the methods just spoken of and the stimulation of chemotherapy. Indeed the direct action of the best known physical and chemical agents is accompanied by a no less important indirect effect recently illustrated by my experimental results, as well as by those of Brancati, Gussio, Caravani, and others, among whom Fränkel and Kimball should be mentioned especially; these have demonstrated an increased resistance in healthy animals suitably radiated before inoculation.

Indirect action deserves the more interest in that there is less definitely a specific factor concerned, the condition reducing itself to a greater sensibility of the neoplastic elements. Their susceptibility subjects them to the general laws of Guilleminot, according to which cells are the more sensitive to radiation the greater their karyokinetic activity, and, therefore, the shorter their vital cycle.

On the other hand, it is noteworthy that doses of radioactive substances only a little greater than those employed, and usually insufficient to cause the disappearance of tumors, are frequently responsible for serious lesions of the body tissues, which, according to the intensity and frequency of the radiation, may pass from hyperplasia to necrosis (Fränkel and Budde, Miller, Heinecke, Unseitig, and Steinhaus).

Thus, radioactive substances exert no specific action on the tumor cell, an impression that has been once again confirmed by Pentimalli. The truth of this statement is evident from the limited destruction of tumor and the extensive damage to healthy tissues produced by strong and penetrating doses. The satisfactory results hoped for through the employment of large amounts have not, therefore, been gained, as not only clinical

observation, but the histological investigations of Aschoff, Steinhaus, Versè, Simon, and Dürk only too clearly demonstrate.

The same may be said of the various chemical substances that have been suggested. For example, the metals which have been reported on by von Wassermann in animals and by Quarelli in man, do not show any specific deposition in the tumor, when normal and pathological tissues from the same organ are compared by extraction, or by polarimetric and histochemical methods. The result is rather that the liver and spleen are generally found to be richest in the substances used. When the quantities introduced are excessive, there are found intense hyperemia, hemorrhage, and degeneration in many organs; especially is this true of the hematopoietic system, as a consequence of which there occur changes in the blood picture (Robin, Ashard, Bardet, Girard, Dugern, de Ario).

On the whole, then, it may be said that the tumor cell has so far offered no specific point for attack by radioactive substances or by chemical agents. Yet in the organs of animals exposed to these measures, structural and functional conditions are encountered which throw some light upon the indirect action of the agents in question.

Thus when too active radiation is employed, or too large amounts of metals, autolysates, or extracts, there are found degenerative lesions and hemorrhages into the parenchyma of the more important organs, and into the tumor itself; and the neoplasm may even be stimulated to increased growth. But when the same measures are applied in suitable doses, a functional hyperplasia supervenes in the various organs, and especially in those appertaining to the hematopoietic system (Fichera, Brancati, Gussio, Caravani, and Szecsi), which is accompanied by arrested development in the tumor or even by its complete disappearance.

Again, in both animals and man, the x-rays or radium in suitable amounts may bring about leucopenia quickly alternating with leucocytosis. In this way one may cause succeeding cycles of leucolysis and abundant regeneration, as has been especially shown by Aubertin, Beaujard, Guilleminot, Manouk-

hine, Murphy, Morton, and Nurenberg. Similar results have been obtained by the injection of colloidal substances which have been found capable of producing effects on the blood-forming organs by von Hanseemann, de Laire, de Ario, Szeesi, and Pentimalli. That the same thing happens after the use of histogenic autolysates is shown by the general condition and by the state of the blood, according to my observations and those of Almagia, Vaughan, and H. Ross.

Under these conditions one may find an augmentation in the size of the liver and spleen, as well as an increase in the function of the blood-forming organs; the latter involves not only the bone-marrow and spleen, but also the liver, even in animals that are no longer very young. These changes, on the other hand, belong especially to the first years of life, that is, to a period in which spontaneous tumors are seldom seen, and are also observed in immune rats, so that they may be considered as an anatomical substratum of resistance (Fichera, Brancati, Goldmann, and Mazza).

The fact, therefore, that these visceral changes are found both in tumor-bearing animals and in normal ones treated by the various agents mentioned above, suggests the possibility of a participation of these functional activities in the regression of tumors.

If organs inhibiting neoplastic development can be rendered more active by suitable treatment, it is not possible to deny that oncolysis may be brought about, indirectly, by their hyperfunction.

In explanation of the lack of response shown by recurrent tumors to the various means of treatment, a possible acquired resistance of their cells has been proposed. But another reason for the difference in behavior between man and animals, or between spontaneous and implanted tumors, suggests itself. The animals used for experimental purposes are young and healthy, and their oncolytic defense is accordingly perfect. They therefore react to stimulation and defend themselves well, whereas in a human being, because of the physiological involution inseparable from age which characterizes the on-

cogenic period of his life, the organs are atrophic, so that the body is less able to respond to stimulation. The increase of this biological disadvantage with time, and with the exhausting demands of treatment, explains the unsatisfactory therapy of recurrences after a previous attempt to cure.

SUMMARY

Certain fundamental principles of treatment may be derived from what has been said:

Importance must be attributed to suitable dosage, to the proportion between the mass to be destroyed and the curative agent, and to the alternation of oncolytic stimulation with various histogenic products. Finally histogenic treatment adds to its indirect action on antitlastic organs an important direct action by reason of the oncolytic substances introduced.

In developing the biological treatment of tumors, chemotherapy should be especially practised by means of autolysates, the importance of which has been emphasized by such authors as Vaughan, C. Lewin, Daels and Deleuze, Theilhaber, Lunckenbein, Koenigsfeld, Rohdenburg, Bullock, and Johnson, Briggs, Bristol, Bulkley, Little, Stammer, Mioni, Citelli, Bauer, Latzel, Wesselly, and Klinger.

For the bibliography the writers quoted in my principal publications should be consulted; from these the present abstract has been made.

REFERENCES

- FICHERA, G: Policlinico, Sez. Prat., 1909, xvi, 1445.
Arch. de Atti della Soc. Ital. di Chir., 1908, i, 437.
Arch. de méd. expér. et d'anat. path., 1909, xxi, 617.
Gazz. d. osp., 1910, i, 526.
Tumori, 1911, i, 1.
Policlinico, Sez. Chir., 1911, xviii, 121.
Tumori, 1913, 2, 410.
Ztschr. f. Krebsforsch., 1914, xiv, 46.
Tumori, 1913, iii, 121.
Tumori, 1914, iii, 760.
Ztschr. f. Krebsforsch., 1914, xiv, 566.
Tumori, 1914, iv, 191.

OCCUPATIONAL CANCER*

H. C. ROSS¹

From Laboratories at the Lister Institute of Preventive Medicine, London

Received for publication September 26, 1917

PART I

The expression "occupational cancer" in reality refers only to a predisposition to the disease, caused by the action of a group of commodities used in certain trades and occupations. The substances in question do not directly cause cancer, but affect the tissues in such a way (usually by giving rise to cell-proliferation of an adventitious type) that the site is rendered specifically prone to malignant invasion. It is well known that soot is one of these commodities, and there is considerable evidence that tobacco-smoke helps in causing the incidence of epithelioma in the throat and larynx. It has generally been considered that this predisposition is due merely to mechanical "irritation" of the tissues, and therefore the subject has not hitherto received much if any systematic investigation, for it is common knowledge that chronic mechanical injury in certain tissues during senescence is liable to predispose to malignant invasion; but during the recent government enquiry into pitch cancer at the briquette works in South Wales some new clinical facts have been found out, which make it appear that some definite, if not specific, chemical rather than mechanical action is concerned in the causation of industrial cancer.² This has led to the investigation of other commodities the use of which also gives rise to cancer under certain conditions; and when all of them are examined collectively, there seems to be considerable

* The author has not read the proof of this article.

¹ Late Director of the John Howard McFadden Research Fund.

² The writer was called upon to give evidence at this enquiry, and it was in connection with it that the following investigations and experiments were made.

evidence, both circumstantial and experimental, to show an association between certain chemical products of death and putrefaction contained in or produced by the commodities themselves and the onset of the disease.

As this evidence is clearly of value in the investigation of the general etiology of cancer, the several commodities will first be discussed *seriatim*, describing the sources and general properties of each substance, the clinical manner in which the disease is brought about, and their power of causing chemical versus mechanical "irritation." Later on the property of producing "smarting of the skin," a chronic dermatitis which is possibly in some instances the initial lesion, and which is an important industrial complaint in some of the trades, will be described. In the description of the commodities references will be given to the description of other commodities, so that comparisons can be made on the one hand as to the incidence of cancer and on the other as to the amount of mechanical injury caused.

It is convenient to take first the coal-tar derivatives and in the order in which they are produced commercially; but this order is inverse to the amount of disease to which the said commodities give rise.

COAL

Coal may be described as a residue of prolonged compression of vegetable matter which has undergone death and decomposition. Coal-miners, heavers, stokers, etc., become covered with coal-dust during their occupations, and yet, in remarkable contrast to its more concentrated products such as tar, pitch, and soot, coal does not give rise to warts and epithelioma. Occasionally a case is reported from one of the mines, but the incidence is rare. In the navy the writer never saw a case among the stokers, and the disease is not specially remarked upon by authorities on miners' diseases.

Mechanical injury. Coal-dust is hard and gritty, and, if it is rubbed on the skin, the fragments are so sharp that they may cause mechanical injury almost amounting to laceration of the epidermis. But coal-dust can be readily washed off the skin.

At the gasworks coal is distilled for the production of gas; at the blast-furnaces it is used as fuel. Bituminous coal is mostly used in England at the gasworks and coke-ovens; for reasons which need not be mentioned here, the harder Scotch coal is mostly used at the blast-furnaces. In both these industries tar is produced as a by-product, and the varieties are generally known as gas-tar and blast-furnace-tar respectively.

TAR

Tar may be described as concentrated coal.³ It is the residue after the first stage in the distillation of coal. By far the greater quantity of it is produced at the gasworks and coke-ovens. Gas-tar varies considerably in its reaction and viscosity according to the temperature of its distillation and to the type of coal from which it is made. Chemically, it contains a large number of substances, some of which will be described later. Blast-furnace tar is very similar except that it is made from a different coal and that it comes over at a lower temperature.

Gas-tar causes warts and epithelioma on the hands and arms of the workmen who handle it, although the incidence is not so great as that caused by pitch or soot (see later). The action of tar is similar to that of crude paraffin, which will also be detailed later. Butlin quoted this action, as described by both Volkmann and Ogston, thus:

Substantially, the effects of the liquid tar and paraffin on the skin of those engaged in the manufacture and whose bodies are actually exposed to them, consisted in eruptions of an acute and chronic character. The acute forms sometimes passed off, and were so completely recovered from, that they scarcely left any trace behind them, but they passed on in many of the people to become chronic. In the acute form the hair follicles and sebaceous glands were chiefly affected, often with the production of an eruption of bright red nodules closely approx-

³ The word "concentrated" is a convenient one to use in this connection as will be seen later, but it should be remembered that, in each stage of the carbonisation of coal and its derivatives, certain fractions are removed; but as the disease caused by each stage is more pronounced, the fractions removed evidently do not concern us.

imate to each other, and usually largest and most numerous on the wrist the dorsal aspects of the parts being most severely affected and the palms of the hands and soles of the feet enjoying a complete immunity. The red nodules correspond with the hair follicles. With the diminution and disappearance of the red eruption, the hair follicle remained enlarged, its mouth gaping and occupied by a little mass of epithelium and dirt, so that the black points were visible over the surface of the affected skin. The only difference in the descriptions of Ogston and Volkmann is that the former speaks of the hair follicles, the latter of the sebaceous glands, as the structures affected and the seat of the black points. In the chronic condition the skin between the hair follicles or sebaceous glands was also altered, it became thickened, dry and stiff, chiefly by increase in the thickness and alterations in the quality of the epidermis. Volkmann describes, in addition (probably because his observations extended over a longer period and were made on a larger number of individuals), little knots of epidermis, tiny horns, and flat, dirty brown scales and crusts. He found that the infiltrations on the skin were most frequent on the fore-arms and scrotum, where particularly they were prone to become moist and offensive.

It is the chronic condition that is liable to become malignant. Unfortunately there is no accurate return of the number of cases at the tar works, for these industries have not yet been scheduled as "dangerous trades," but from enquiries from the tar-distillers, cases of malignant invasion of the warty skin are not uncommon.

Blast-furnace tar, on the other hand, seems to be harmless.

Mechanical injury. There is an absence of fine particles or grit from tar, which is a viscous liquid at ordinary temperatures. In the strict sense of the term, therefore, it can hardly be accused of causing *mechanical* irritation, but when it dries on the skin, especially after vigorous washing, a little mechanical injury may be caused before it can be entirely removed from the pores.

Both gas-tar and the blast-furnace variety are consigned to the tar-distillers, who, by further carbonisation, obtain various commodities from it, such as ammoniacal liquor (sulphate of ammonia for manure), naphthas, creosotes, phenols, anthracene oils, and many others.

The residue is the black bitumen, which sets on cooling, known as gas-tar pitch and blast-furnace pitch respectively. Four-fifths of the pitch made is consigned to the briquette works.

PITCH

Pitch may be described as concentrated tar. Gas-tar pitch causes the disease known as pitch warts, which is fairly frequently followed by pitch cancer. The common site, as in the case of soot, is in the scrotum, but the warts also appear frequently on the face, neck, and lip; next to the scrotum, the lip is the commonest site for malignant invasion of the pitch wart. From time to time opinions are advanced that pitch cancer is not true cancer; but they can be disposed of by the fact that the disease is identical with chimney-sweeps' cancer, and has within the last year to the writer's knowledge caused at least two deaths in Swansea alone.⁴ It is true that the warts are not malignant at first, and there is evidence to show that they do not become malignant, in a manner similar to the case of tar, until ulceration has occurred, leaving an open sore at the base; but once they become malignant and remain untreated, the disease runs its usual course with metastasis and death, although, as the lesions involve the skin, the mortality is not very high owing to early detection and the facility for complete removal.

At the briquette works, pitch is ground into a fine powder and mixed with small coals, the mixture being damped by steam and compressed into the blocks which constitute the "patent fuel." The pitch grinders suffer most, although there are some cases among the men who handle the briquettes. Out of 246 men examined by Dr. Legge, H. M. Inspector of Factories, 51 suffered from pitch warts and 28 showed signs of old ones. It is probable, as Mr. Commissioner Lush (1) has pointed out, that the incidence is higher than this, as the numbers examined included men other than the pitch grinders. In the writer's experience at the works, the incidence of pitch warts among the grinders is practically universal except in the cases of boys or young men.

⁴ 1914, since then the disease has frequently occurred.

It is remarkable how pitch warts become more common in the men as age advances, a point brought to light at one of the Home Office conferences with the briquette trade, and apparently in harmony with the etiology of tumors in general. The actual clinical condition cannot be better described than by the following quotation from Dr. Legge's Report (2), in which it is pointed out that the men engaged in the manufacture of briquettes

are liable to suffer from warty growths, which ulcerate and occasionally become the seat of epitheliomatous cancer. The growths may occur on any part of the body, and are common on the face, hands, and scrotum. [The region where the collar rubs is a very common site.—H. C. R.] They commence as small nodules in the skin and almost immediately begin to break down, forming an ulcer covered by a crust, which gives the characteristic appearance to the so-called wart. The underlying ulcer almost invariably heals up, leaving a small scar when the crust has fallen off. We believe this to be the normal course, although witnesses regard the scar as the result of the primitive methods of treatment adopted by the workers. When they take on an epitheliomatous character the situation is almost invariably on the scrotum. The disease then follows the usual course, involving neighboring organs and tissues and can only be arrested by free excision. Epithelioma is the least malignant form of cancer, and on removal is not usually followed by recurrence. We saw men still at their ordinary work and in good health, on whom such operations had been performed several years previously. The amount of incapacity caused by the condition, whether in its benign or malignant manifestation, need not be great. There was general consensus of opinion that the cancerous conditions could be prevented by scrupulous cleanliness, but we satisfied ourselves that *occasionally* it may develop in those who have paid all reasonable attention in this respect.⁵

Blast-furnace pitch does not appear to cause either warts or cancer (1), (2). The difference is a most important one; and the government, in imposing regulations (1) to deal with pitch cancer, specifically exempted those factories in which only blast-furnace pitch is used. Yet the two forms of pitch are

* The italics are mine—H. C. R.

practically identical except that the blast-furnace variety is made from tar which comes over at a lower temperature, and which is mostly made from the less bituminous Scottish coal.

Mechanical injury. Both forms of pitch are gritty, but less so than coal and more so than soot. At the hot temperatures of the briquette factories, ordinary pitch softens slightly on the men's skins, when it is sometimes difficult to remove; blast-furnace pitch, however, does not so easily soften.

SOOT

This substance, which may be described as concentrated pitch, is the best known cause of industrial cancer. The mortality from it among chimney sweeps is twice what it is among occupied males generally. The mortality in 1900-1902 was 133 as compared with 63 among occupied males at the same age. "This excessive prevalence is certainly due to the nature of the trade, the soot setting up an irritation of the skin similar to that produced by pitch or tar and with similar results." (1)

The form of soot that gives rise to this disease is that produced by the carbonisation of coal; and more soot is set free by burning bituminous coal than by the use of the harder varieties, such as anthracite. Soot,⁶ however, is not pure carbon but also contains tar and tar oils with a certain amount of varying degrees of pitch. It is not gritty as a rule, but of a soft floury nature due to the tar oils, as can be readily demonstrated by placing some on blotting paper, when the oils will produce a greasy exudation. Owing to this oily nature, soot can only with difficulty be removed from the pores of the skin; but although the sweeps' bodies are covered with it from head to foot, like pitch, it causes the disease practically on the scrotum only—preceded, as usual, by warts. This is a remarkable fact, but at the same time it should be remembered that with this class of workmen,

⁶ "There is 'sut' and 'sut,'" an expression frequently heard at tar and briquette works. Soot is the cause of chimney sweeps' cancer and is collected from domestic chimneys. "Sut" is practically clinker, consisting of carbon and earthy salts, like the smoke-box ashes produced in locomotive flues. High temperature and pressure furnaces produce the latter, which is harmless.

who rarely have baths, the scrotum is the dirtiest part of the body, and is constantly subjected to rubbing from dirty corduroy trousers. Moreover it is a part where sweating is profuse.

Mechanical injury. The absence of grit is an important point, because the flouxy soot differs in this respect very materially from coal-dust, which does not cause cancer at all, yet which will cause mechanical injury amounting to laceration.

We may now sum up the foregoing commodities to show the relative amount of mechanical irritation caused by each, comparing that with the amount of cancer caused by each.

MECHANICAL INJURY	COMMODITY	CANCER INCIDENCE
Greatest	Coal-dust	Nil
Considerable	Blast-furnace pitch	Nil
Only little, as it softens	Gas-tar pitch	Considerable
Practically nil, a liquid	Tar	Several cases, but less than pitch
Nil	Soot	Greatest incidence of all

It seems clear from this clinical evidence that pitch warts and epithelioma in the coal-tar industries are not due merely to mechanical irritation, for the more the irritation the less the disease.

Since the predisposition is not due to mechanical injury, it is obvious that it must be due to chemical injury, which is borne out by the fact that it is the most concentrated form of coal, namely soot, which causes the most disease, the incidence among the intermediate trades rising in exact ratio with the concentration; thus, coal causes no warts or cancer, tar causes some cases, pitch causes many more, and soot causes a double mortality. The gradual concentration of the coal, therefore, also apparently concentrates at each stage of the distillation some substance which seems to be the predisposing cause of cancer in all these industries.

That some chemical factor is concerned is also evinced by the harmlessness of blast-furnace tar and pitch. These substances cause more mechanical injury than their gasworks prototypes,

and yet they have no cancer-producing action whatever. But the blast-furnace tar is produced at a lower temperature and from different coal, which makes it all the more probable that the mischievous chemical substance is less effective or absent from this commodity.

ANTHRACENE FRACTION OF TAR

There is a theory at the works that the mischievous substance is contained in the anthracene fraction of tar, because of recent years it has not paid, as formerly, to remove this fraction for the manufacture of dyes, and the leaving of it in the pitch coincides with the increase of the disease in the last decade or two.

NAPHTHALENE

It is also clear that the products of the lighter fractions are not to blame, because those who make boot-blackening and other "blacks" do not suffer. Boot-blackening is made by the carbonisation of naphthalene (isolated from a tar fraction lighter than the anthracene fraction) which is converted into a "soot" by burning on hot plates. The workmen in this industry become covered with this "soot," but Dr. Legge states that there are no cases of pitch warts or cancer among them. This is another piece of evidence against mechanical injury, for naphthalene soot and coal soot are similar as regards the physical nature of the particles, yet one is harmless and the other most dangerous.

OTHER TARRY COMMODITIES

Liquor carbonis detergens, creolin, etc., are used extensively on the skin. In some cases they cause thickening. They seem to promote the cell-proliferation of healing, and are used under prescription for this purpose. They contain the middle fractions of tar, which seem to be responsible for the proliferation which leads to the warts in the industries.

In this respect it may be useful to draw attention to the tar capsules which from time to time are advertised to be taken

internally as an aid to digestion. Those that have been tested in these laboratories also contain, as a rule, the middle tar fractions, and therefore it would appear advisable for them to be prescribed only with great caution. The intestinal epithelium is much more liable to malignancy than that of the skin, and subjecting it to a specific commodity is to be avoided.

There is no evidence that dust from the tarred roads causes warts, but there have been some complaints of conjunctival inflammation from it.

"SMARTING" OF THE FACIAL SKIN DUE TO PITCH DUST

This point may be conveniently discussed before we leave the tar products, although it is doubtful whether it is connected with the pathological changes which lead to pitch warts. As Mr. Lush points out (1), all fuel workers suffer from a burning sensation in the face especially when exposed to keen winds or sunshine, and one of the chief objections to the bathing regulations was that washing made this condition worse. The physical signs are not very noticeable; sometimes there is slight dermatitis with a little peeling, but never any discharge. There is never a "flare up" like that seen in eczema. The burning sensation is undoubtedly troublesome, coming on sometimes after an hour's exposure to the pitch dust—as the writer can personally testify—and it may last for several hours. The condition seems only to affect the cheeks and the region behind the ears, and it may be so bad as to prevent the men attending football matches.⁷

Some cargoes of pitch cause it more than others, and the bad varieties seem frequently to stain the skin a yellow color.

Experiments were made at the works to find out the cause of this burning of the skin. Some pitch was finely ground and then thoroughly washed with water and dilute acid; the water was allowed to dry on the skin, but no burning sensation could be felt. Two of the pitch grinders then rubbed the washed pitch

⁷ More recently this or a similar burning of the skin has occurred to a considerable degree among munition workers who handle certain coal-tar explosives. An acute dermatitis is produced, with a yellowish discoloration of the skin. The question is being investigated.

powder on their skins, when it was found that it still caused the dermatitis. It is apparent, therefore, that this burning is at least due in part to slight mechanical injury. A watery extract from some of the pitch which was more irritating than that from other cargoes, and which contained the yellow ingredient, did, however, cause a little smarting when applied to the dorsum of the hand, and it is possible that this is due to an excess of acridine.

But the common sites of the smarting are not those of the pitch warts; the lips, eyelids, and scrotum are not affected. It is possible that the slight mechanical injury may leave fissures in the skin in which the pitch dust may become impacted, and so lead to pitch warts, and the swarthy skin, which is due to "blackheads;" but the part played by it, for the reasons given above, is probably a minor one.

Attempts to protect the men's faces by fullers' earth was partly successful, but the men refuse to use it; protection by liquid paraffin caused such acute erythema that it had to be abandoned.

There is little evidence that smarting of the skin occurs among coal miners; soot appears to cause it only to a small extent. Dr. D. A. Crow has kindly interrogated 36 sweeps on this point, and has elicited the fact that 26 of them complained of slight burning, one saying that boiler soot from coke was particularly noxious. It may be due to the sticky nature of softened pitch and soot, which may necessitate tearing of the epithelium when washing it off.

PITCH FROM PITCH LAKE, AND FROM MANJACK MINES, TRINIDAD, WEST INDIES

Dr. H. L. Clare, the Surgeon General, Trinidad, has been good enough to supply the following information.

The Mines (Manjack) near Princes Town have not been worked since the beginning of the war. I have never heard of any unusual—or indeed of any incidence of cancer amongst the laborers; these mines are worked at a considerable distance below the surface, while the Pitch Lake work is all on the surface.

Dr. George Campbell, Resident Medical Officer of the Pitch Lake Company, reports to Dr. Clare, thus:

I have had no case of cancer among the pitch workers here during the last five years, and can find no history of such a case having occurred. I may say that the workers come very slightly into contact with the pitch, the actual process being largely one of cutting and loading. The pitch of the lake does not come from bituminous coal, but is closely related to the non-volatile constituents of crude oil, with no paraffin base. Composition: water and gas 29 per cent, bitumen 39 per cent, organic matter 7 per cent, mineral matter 25 per cent. Composition of the bitumen: carbon 82.33, hydrogen 10.69, sulphur 6.16, nitrogen 0.81.

The small content of nitrogen is most interesting, as will be seen later. The fact that the pitch is not bituminous is also worthy of note.

ANILINE DYES

There are practically no manufactories of aniline dyes of a large size in England, and it was difficult to obtain reliable information about industrial disease on the Continent, even before the war.⁸ The Chief Inspector of Factories, however, refers to a series of 14 cases of villous growth of the bladder in a works where the men were engaged in handling aniline dyes made there. Dr. Legge's report of February, 1911, also states that

Dermatitis and eczema are produced by contact with several of the aniline colors—chrysoidin, Bismarck-brown, paranitranilin red, and others, but in none so markedly and so certainly as in the manufacture of dinitrochlorbenzene. In all the 16 cases referred to, dermatitis of the face, arms, armpits, and such parts of the body as are likely to be bathed in sweat, was the characteristic symptom, unaccompanied by any noticeable changes in the blood.

⁸ The late Sir Henry Butlin, President of the Royal College of Surgeons, journeyed to the Continent expressly to ascertain the incidence of pitch-cancer at the briquette works. He returned with the information that the disease does not exist. Enquiry "through the trade" however, shows that it is about similar to the incidence in Great Britain.

PETROLEUM

There is no information that dermatitis or cancer is caused at the petroleum wells in America, Russia, or elsewhere; but absence of information does not necessarily mean that disease does not exist. At the shale oil^a works in Scotland, however, paraffin dermatitis and cancer are not uncommon. Volkmann and Ogston's description, already quoted (see tar), also refers to the effects of paraffin. The two substances seem to produce very similar results. To judge from enquiry at the works, it is the crude paraffin which causes the trouble, and there have been some cases in the oil produced from cannel.

In his annual report, Dr. W. Walker, certifying surgeon for Mid Calder, states that during the year he has come across a few cases of "paraffin cancer" a disease from which shale oil workers are peculiarly liable to suffer, some more than others. It begins in a variety of forms, i.e., as an erythema, pimples, papules, etc.; these gradually dry, leaving hard crusts which, increasing in size, form hard, elevated, wart-like masses. As they increase in size these break down, and produce sloughing ulcers or paraffin cancers. He mentions three cases: (1) On the dorsum of foot, doing well after operation; (2) scrotal, so far doing well after extensive operation; and (3) back of wrist, necessitating amputation of the right arm at the shoulder. Enquiry by Mr. Brown (Edinburgh) elicited the following information as to the process of refining, so far as it affects the material handled in the press house, and as to the effect on the skin.

The shale is heated in vertical retorts, the gas being condensed, and the ammoniacal liquor separated from the oil by gravitation. The crude oil is distilled by heating and blowing steam through it, the result of the distillation being known as "green oil." The green oil is treated with 2 per cent sulphuric acid, and afterwards, in another vessel with 1 per cent caustic soda. The green oil already treated is again distilled with steam and fractionated, giving "light oil," "intermediate," and "heavy oils," the latter oils containing paraffin. The

^a Shale and cannel are forms of coal.

light oil is treated with sulphuric acid and caustic soda, and then distilled, the result being "burning oil" which is ready for use, and "intermediate oil," which is added to the intermediate oil already obtained by the previous distillation of the green oil. The "heavy oil" passes through pipes to the cooling sheds, and is pumped through ammonia freezers and filter presses, the residue, in the form of a sludge or scale, being conveyed by a worm screw to the floor of the hydraulic press house. The heavy oil from the filters is re-treated with sulphuric acid and caustic soda, and again distilled and separated into "intermediate oil" and "lubricating oil." All intermediate and lubricating oils are then passed through coolers, freezers, and filter presses, and the scale is conveyed to the floor of the hydraulic press house by worm screw. It is this scale which causes the eruption on the arms of the workers. The men shovel the scale on to canvas cloths, which are laid in layers on bogies, the ends and sides of the cloths being folded over and the pile placed under the hydraulic presses. The liquid oil is pressed out and returns to the process again. The paraffin scale remaining in the cloths is shaken by hand into a melting pot, and blown into trays in sweating houses where it is purified by slow heat. It is then treated with animal black and is finally converted into wax. The men in the hydraulic press houses wear canvas bibs and leg coverings, but their arms are bare to the shoulders, and in handling the cloths they cannot escape coming into contact with the scale. The eruption usually takes the form of small pimples or red blotches, and is not painful unless these develop into boils. I examined the arms of 10 men of whom five showed warts, pimples, or scars.

The higher fractions, such as paraffin wax and "liquid paraffin" seem to be harmless. Indeed, liquid paraffin is consumed internally in enormous quantities as a purgative, and since its introduction as such during the last ten years there has been no such marked increase in intestinal cancer as one would expect if this substance caused a predisposition to cancer like pitch or soot. The higher refining process therefore evidently removes some noxious constituent.

Crude shale oil from some mines evidently cause more trouble than that from other mines, for several cases are reported from certain districts and none from others.

At the International Congress of Medicine in London, in 1912, Dr. Norman Walker showed some beautiful casts of paraffin dermatitis and epithelioma.

VASELINE

This is a petroleum fraction. It is used extensively on the skin to aid healing, and is said to increase the cell proliferation of healing.

GREASE

The manufacture of grease causes complaints similar to those produced by tar, but it requires no special comment here because they are evidently due to the tar oils which form a large constituent of commercial grease. Dr. Legge examined 31 men at grease works and found 6 suffering from pitch warts and 13 who had signs of old ones.

KANGRI CANCER

The "fire pot" is a wicker brazier having an earthenware center used by the natives in certain parts of India. It is carried inside the garments, as a rule suspended by straps over the shoulders. The fuel usually consists of decayed vegetable matter which forms a charcoal. The continual rubbing of this charcoal against the skin of the abdomen and thighs gives rise to thickening, ulceration, and epithelioma of the skin in much the same way as coal-soot and pitch.

TOBACCO

Smokers' cancer of the lip is generally associated with mechanical injury caused by the rough stems of clay pipes, and the incidence is less than it was owing, apparently, to the introduction of vulcanite stems; but mechanical injury will not account for the high incidence of malignant disease of the larynx and fauces of men as compared with that of women, in whom this disease is rare. Tobacco, as it is smoked, consists of the partially dried leaf which has been allowed to ferment by putre-

faction. It is frequently mixed with olive oil (a substance which causes dermatitis in several trades), and is sold damped to various degrees with water. The drier the tobacco the more the smoke is inclined to "burn" the tongue.

BETEL NUT

The so-called betel nut that is chewed by natives in Madras, Ceylon, and the Straits Settlements is a mixture of betel nut, tobacco, and spices, which is allowed to remain in a warm place until it becomes putrid. It is the cause of epithelioma in the floor of the mouth.

X-RAYS AND RADIUM RAYS

Radiation need only be mentioned here as causing burns of an intractable nature. They lead to ulceration which is liable to malignant infiltration. So much has been written on this subject, that passing mention only need be made.

ARSENIC CANCER

Pye-Smith (3) has recently published a collection of 31 cases of this disease, giving an accurate description of each. He points out that in nearly all cases of arsenic cancer

It looks as if arsenic induces keratosis of the skin (as is now universally admitted), the keratosis mechanically inducing fissures, which readily become infected with bacteria and then ulcerate, the ulcers finally becoming cancerous.

He also suggests that arsenic may predispose the tissues generally towards cancer, a predisposition comparable to or even more marked than that caused by senescence; but it may be remembered that arsenic is a powerful cell-poison, and it may therefore bring about a result similar to that produced by old age, by causing cell-death.

It has been suggested that pitch cancer may be due to arsenic in the pitch. Tests made in these laboratories, however, have

failed to detect any arsenic in pitch. The pitch used at the briquette works has been raised to a temperature of 300°C. before it is consigned there. The same remark applies to the suggestion that pitch cancer is due to turpentine. There is no turpentine in tar, pitch, or soot.

LEUCODERMA, KERATOSIS, AND CANCER

Lenthal Cheatle has drawn attention to the frequent incidence of malignant infiltration supervening on patches of atrophic skin which have become subsequently thickened. These areas may even be bilateral, and may in the first instance be caused by nerve disease. As in arsenic cancer, there seems to be a local destruction of cells followed by proliferation, which may ulcerate and ultimately become cancerous.

FOUL ULCERS

Old untreated varicose or syphilitic ulcers are prone to become malignant, as is well known. Since there are no figures available, it is difficult to ascertain with certainty whether the degree of suppuration does or does not increase this liability; but it seems common experience to find the malignant infiltration in the more neglected cases.

MANURE

Senile warts occur on the hands and arms of farm laborers who frequently handle manure. The Registrar-General's returns for 1890-1892 show an incidence of 84 deaths as against 51 for all occupied males. In Germany agricultural laborers are especially subject to skin cancer.

When the foregoing commodities are examined collectively it becomes more apparent that mechanical injury per se can play only a minor part in the predisposition to occupational cancer. Apart from the evidence in this respect given by the coal-tar industries, the action of petroleum, arsenic, manure, tobacco, etc., also demonstrates it; for some of these commodities are solids, some are liquids, and physically they differ a great deal

in other respects. Yet they all seem to bring about the predisposition to malignancy in a somewhat similar manner, namely, by becoming impacted in the tissues, where, by giving rise to cell proliferation, they produce a warty condition. The warts usually ulcerate at their bases and drop off, leaving an intractable sore. It seems to be the edges of the ulcers that become epitheliomatous, and there appears to be a specific liability, when the industrial warts are untreated, for this to happen.

Mechanical trauma, it is true, will predispose the tissues to malignancy, both carcinoma and sarcoma, a fact proved by clinical experience and by experiments on animals; but in occupational cancer it is clear that a chemical factor is also concerned which acts directly on the tissues, in the first instance inducing cell proliferation. If it were not so, apart from the evidence enumerated above, we should expect to find a similar incidence among chimney-sweeps and briquette makers as among stone-masons and metal-grinders, who work with even more gritty commodities, and yet who do not specially suffer. There must be a chemical factor responsible and the question is, what is it? It is true that *x*-ray cancer and the predisposition caused by scars, keloid, and degenerate nerve areas may be initially due to mechanical trauma, that is to say, to excessive energy or heat, or destruction following upon deficient blood supply, etc.; but these factors are not sufficient to account for the incidence of occupational cancer, and it is possible that the initial trauma in *x*-ray cases may also be followed by some specific chemical change in the tissues.

In conclusion, one further point may be mentioned. When the active commodities are examined collectively, it will be seen that all of them, with one exception, are products of, or are derived from, the products of the death and decomposition of living matter, although such death may have occurred centuries previously. Coal and its derivatives, including paraffins, charcoal, tobacco, betel nut, and manure, all have such a derivation; the one exception is arsenic, and that, being a powerful cell-poison, may possibly produce the products of cell death and decomposition in the tissues. It is important to bear this in

mind, although there is no actual proof that such products predispose the tissues to epithelioma; yet it is a striking point, for these commodities undoubtedly cause this predisposition when impacted for a long time in the tissues of the skin, whereas inorganic commodities, such as stone-dust and metal-dust, are ineffective. In this connection, it is useful to remember that carcinoma seems more frequent, in general, at sites which are continually subjected to organic matter undergoing bacterial decomposition, such as the rectum, stomach and indeed the whole intestinal tract), mouth, uterine cervix, breast (chiefly in women who have borne children), prepuce, anus, scrotum, etc. In sarcoma, on the other hand, this does not apply, any more than does the age incidence; but with occupational epithelioma especially, given that the predisposing cause of the disease is present equally in different parts of the body, it is usually in that part which is the dirtiest or which is affected with open ulceration that the malignant condition supervenes.

Decaying organic matter of all descriptions fertilizes living matter, and promotes cell proliferation, being used extensively for the growing of plants in agriculture. Up to a certain point, the more putrid the matter, the more pronounced is this fertilizing property. Soot is used by gardeners for this purpose, but it is only useful when it is wet, showing that it is necessary to dissolve something out of it, namely nitrogenous compounds. Mere grit has not this action. Ammoniacal liquor distilled from tar is a very valuable commodity, owing to the nitrogenous compounds it contains, being converted into artificial manures. The trade in nitrates for fertilization is enormous. Soot and tar products, in causing the cell proliferation leading to warts, would seem to act in accord with this principle, and epithelioma itself is also a growth consisting of an excessive cell proliferation.

To sum up: There seems to be some specific *chemical* agent responsible for occupational cancer; *mechanical* irritation plays only a minor rôle in this disease; and lastly, there is a probability that the mischievous agents are organic nitrogenous substances of a group common to all the dangerous commodities. In the coal-carbonisation—commodities, the fact that the coal is bituminous seems to be essential.

PART II

If one were to attempt to isolate a substance or a chemical grouping common to them all from the many and complex commodities that give rise to occupational cancer, one would be faced with a problem so vast that it would be futile to undertake it. The coal-tar derivatives alone would present an insuperable problem, for they contain a large number of substances of many varieties, including nitrogenous bodies of several descriptions. Besides, there would be nothing to go upon; one would not have the faintest idea how to start. One might fractionate gas-tar, pitch, or soot, but even then one would probably hit upon the wrong fraction and it would be impossible to correlate such a chemical analysis with a subsequent one made on tobacco-smoke, betel nut, manure, and any various compounds of arsenic which may in some way be produced by the drug in the living tissues. It may be remembered that arsenic is a "cumulative" drug.

After consideration it was resolved to attack the question from a point of view based on a working hypothesis. It is admitted that this principle of making facts—such as those given in the first part of this paper—harmonise with even a working hypothesis is illogical; but there was no alternative. The only way was to choose a reasonable line of argument, keeping all the facts known about occupational cancer and cancer in general always in view, and then to proceed experimentally.

First the facts known about cancer in general were reviewed, and then the more acceptable of the several theories and working hypotheses were studied to see how far they could be made use of in the investigation of occupational cancer. Some of the facts have already been alluded to in the first part of this paper, e.g., the remarkable age incidence of carcinoma (the form of disease with which we are immediately dealing), the common sites, the question of chronic mechanical irritation, and above all the phenomenon of excessive cell-proliferation which is a characteristic of malignant disease. But two other facts were also kept prominently in view, namely, the propensity

on the part of the cells to infiltrate surrounding tissues, and the extraordinary fact that if the disease remains untreated the subject of it emaciates and dies for some reason that has never been satisfactorily explained.

The clinical data given in the first part of this paper undoubtedly justify the conclusion that, when dealing with the cause of occupational cancer, we are in reality concerned with two factors—a *predisposing* cause and an *exciting* one. The predisposing cause is evidently a chemical derived from the commodities when impacted in the tissues, but the exciting cause seems only to appear when ulceration has occurred, especially if the ulceration occurs in the dirtier parts of the body.

The term ulceration, when used in this sense, is not a very satisfactory one, for, although the classic definition "solution of continuity of surface" seems always to hold good before the site becomes malignant, the epitheliomatous condition invariably appears at the growing (cell-proliferative) edge where there may be no immediate loss of surface continuity, and the demarcation between the benign and malignant proliferation may not be clearly defined.

But there is a predisposing cause and an exciting cause undoubtedly at work before a case of occupational cancer becomes truly malignant. The specific commodity impacted in the tissue giving rise to cell-proliferation like a wart; sloughing, leading to ulceration; more cell-proliferation at the edge with ultimate infiltration, metastasis, and death. A definite sequence of events.

On the face of these facts, therefore, the older theories did not help very much. Take for instance the general hypothesis that cancer follows on chronic irritation, in favor of which there is considerable evidence. Let us analyse the expression "chronic irritation." An irritant must be either mechanical or chemical; a mechanical irritant must be due either to pressure or heat, or to both. The hot stem of a clay pipe is an example; an ill-fitting boot causing a corn which may become epitheliomatous is another. What happens to the individual living cell in the tissue which is subject to mechanical irritation? The term

"irritation" really is a clinical one; it has not a precise meaning to the cytologist. To the clinician the word implies a degree of injury as evinced by some reaction such as inflammation, pain, or itching, but there is no means of measuring the amount of injury suffered by the individual cell. Chronic irritation in a tissue is a definite condition, but when we discuss the individual living cell we had better use the generality—injury. "Injury," surely, is the right word, because both heat and energy, if present in excess, will ultimately produce damage and even death (destruction) in a tissue. Irritation is merely a degree of this injury. So it must be with the individual cell. The cause of the irritation, as it increases, must sooner or later first damage and then kill the individual cells which constitute the tissue and which cannot run away. It is submitted, therefore, that when we speak of chronic mechanical irritation in a tissue, we are in reality dealing with a population of cells, some of which are normal, some damaged, and some dead, according to the degree of injury. If the chronic irritation is slight, there may be no death rate above the normal, but there may be damage to a certain number of cells. Presumably damage to a cell means death of portions of its protoplasm. The pathological term "degenerate cell" usually means vacuolation when it is examined intra-vitam.

A chemical irritant seems to fall into a similar category. Chemical irritants, if their administration is pressed, sooner or later become destructive agents. The word "irritation" here again means a degree of injury caused to the tissue by the irritant, namely damage or death to the individual cells as the case may be. If a substance is an "irritant" in certain dosage, it must become poisonous in greater dosage. A given chemical in given dosage can only have one of three actions on the living cell; it may be beneficial, inert, or injurious, i.e., poisonous. The chemical irritant must be a degree of the poisonous variety.

As regards *mechanical* irritation: The first part of this paper has shown conclusively that mechanical irritation per se cannot be responsible for occupational cancer; the coal-tar commodities alone prove the point. A specific chemical is concerned without

doubt. Is this chemical an irritant? is the next question. Obviously not, for it produces cell-proliferation, not destruction in the tissue.

Moreover, if occupational cancer were entirely due to chemical *poisons* derived from the commodities, we should certainly expect, for instance, a greater incidence of the disease among those who handle the lighter fractions of coal distillation, especially the phenol, creosote, and naphthalene fractions, than among those who handle tar or pitch. But these lighter fractions, although cell-poisons, are harmless as far as the predisposition to cancer is concerned. A striking contrast to the non-poisonous soot. Surgeons and nurses, whose hands are frequently immersed in concentrated solutions of mercury biniodide do not suffer from cancer of the hands like those who are employed in handling paraffin at the shale works. Yet the mercury salt is infinitely more poisonous than anything contained in petroleum.

Trinitrotoluene and similar coal-tar derivatives used in munition manufacture, when impacted in the skin, cause dermatitis, and even atrophy of the liver, owing to their poisonous effects. Yet there has been no "flare" of squamous-cell epithelioma as a result of their action.

Nevertheless, apart altogether from occupational cancer, it must be admitted that chronic mechanical injury *does* give rise to a predisposition to malignancy, to wit—the clay pipe and the lip, the ill-fitting boot and the corn, the blow on the breast and scirrhus, the operation on the mole and melanotic sarcoma. Chemical injury also, in a similar manner, apart from occupational cancer, undoubtedly predisposes tissues to carcinoma, namely, chemical substances produced by inflammation as occurs in an erosion of the uterine cervix, syphilides of the tongue, etc.; these seem frequently to be due to chemical irritants of some degree.

But irritants are not nearly so powerful in their action as the occupational commodities. Neve, of the Kashmir Mission Hospital found 848 cases due to Kangri soot out of a total of 1189 squamous carcinomas. It is true that arsenic is a cell-

poison, and therefore might be classified among the "irritants," but the predisposition due to arsenic does not appear until years have elapsed after the patients have taken and have ceased to take the drug.

Injurious agents, therefore, both mechanical and chemical, by virtue of the degree of irritation they produce, by causing an amount of damage or death in the cell-population, may produce in a tissue a predisposition to cancer; but in occupational cancer something more is needed, namely a specific chemical which is contained in all the commodities themselves, and which sets up cell-proliferation (the wart) when impacted in the tissues.

The time honored hypothesis of Cohnheim does not harmonize with the clinical facts regarding occupational cancer. One could imagine, perhaps, that certain cells might retain a sort of quiescence from fetal days and then "light up" during senescence, although why these cell-rests, which are physiological components of the body, should ultimately destroy the patient, and incidentally themselves, is difficult to understand. One could even imagine that chronic mechanical injury might in some way cause the cell-rests to "light up," but it is impossible to believe that those who grind gas-tar pitch in Cardiff should have cells included from birth, while those who grind blast-furnace pitch in Whitehaven should enjoy immunity. Cohnheim's hypothesis drives us to this *reductio ad absurdum*.

The same argument applies to the suggestion of Farmer, Moore, and Walker, which is based on the Weismann hypothesis of inheritance, and which, like Cohnheim's, does not divide the cause of cancer into predisposing and exciting origins. It is by no means proved that the cells of vertebrates are separated into somatic and reproductive varieties, and, even if that were the case, it is not probable that Indians who use the Kangri fire-pot should have more reproductive cells in the skin than those who become covered with shoe-blackening.

Shattock and Dudgeon have lately sought to advance the question by attacking the cause of cell-proliferation. They have suggested that cells multiply instinctively unless restrained by an antibody, and that pathological variation in the antibody

may produce tumors, etc. An antitoxin for an instinct is decidedly new; but as a solution of the problem of growth, the very premises of this hypothesis have never been proved; on the contrary, some of the earlier experiments at Rothamsted Experimental Station have shown that plants, at least, become barren if they receive no chemical stimulus from without.

The work of Roger Williams is of more assistance, for, apart from the mass of information on cancer in general contained in his book, he draws a suggestion from it that the immediate nutrition of the cells may play a rôle in the causation of the disease. The conclusions arrived at in the first part of this paper appear to be somewhat in accord with this view.

On the whole, therefore, the well known hypotheses did not offer a firm standpoint on which to base a series of experiments. None of them offered any test to which the several commodities that cause occupational cancer could be subjected. So it was considered better to proceed on lines of work in which the writer had been engaged for several years, investigating the causes of cell proliferation. As pointed out in the first part of this paper, cell proliferation is the first and a constant result of the action of all the commodities which give rise to occupational cancer.

This working hypothesis was based on a series of experiments which has produced evidence that, so far as certain groups of cells at least are concerned, their division is due not merely to instinct on the part of the cells themselves but to specific chemical agents, all of which appear to be amino substances set free by cell-death. According to this hypothesis cells divide in response to this *stimulus* from without; that is to say, the reproduction of cells is initially prompted by death occurring among their neighbours, cell-death setting free the amino substances, and the latter exciting cell-division among the surviving cells.

The hypothesis arose in 1909 from research with the jelly method of in vitro staining, by which it was found that cell-division can be induced in living human lymphocytes by mixing watery extracts of dead tissues with the jellies on which the individuals cells are resting. Subsequently the extracts were made to induce division in leucocytes and certain epithelial cells also.

Improvement in the test revealed the fact that the active agents in the watery extracts were nitrogenous substances produced by the decomposition of proteins, and several were isolated and proved to consist, among others, of some of the purin bases, such as creatin, xanthin, and tyrosin. For convenience the name *auxetic*¹⁰ was given to these cell-division-producing agents.

The action of auxetics on blood-cells was confirmed by R. Ross and again by Drew. Fantham succeeded in inducing division by auxetics in individual *Entamoeba coli*, the writer in the ova of *Ascaris megalocephala*, Drew in the spores of *Polytoma granulosa*, and in a fine series of experiments Cropper and Drew (4) have found that amoebae increase enormously in numbers in response to auxetics. Indeed the last authors have found that when individual amoebae are isolated from all other living creatures and placed in distilled water or on jelly containing only distilled water, they will not divide at all unless an auxetic be present, that auxetics will up to a certain point increase their proliferation according to the strength of solution, but that after a few generations have passed the presence of a ferment is also necessary if proliferation is to be kept up.

With these various classes of cells, then, auxetics undoubtedly prompt proliferation. When first the experiments with human white blood cells were published, it was disputed that the figures induced in lymphocytes were those of cell-division, because the jelly method revealed the fact that these figures, although they were in general principle those of mitosis, as far as lymphocytes are concerned, were dissimilar as regards what is generally believed to be the nucleus. But estimations of increase in numbers by precise methods, together with the work of Cropper and Drew and others, seem to settle this point, for the cell-division which they induce in amoebae is of the normal type.¹¹

Since certain human and some other cells will divide in re-

¹⁰ The name implies an excitor, which in its turn may and has been interpreted to mean a stimulant; but it is debatable whether they are such or whether they are foods. Without doubt, some are foods.

¹¹ Cropper and Drew's work has recently been confirmed by H. G. Thornton and Geoffrey Smith using *Euglena viridis*. Proc. Roy. Soc., Series B, London, 1915, lxxxviii, 151.

sponse to auxetics, and since these auxetics are produced in a tissue by cell-death, the working hypothesis was extended to cell-proliferation which is frequently the predisposing cause of cancer. Injury gives rise to cytolysis and to the cell-proliferation of healing, and if it becomes chronic, proliferation to the extent of production of benign tumor formation may be brought about. In order to test the last point, auxetics were introduced into the mammary ducts of goats' breasts—using a blunt hollow needle to prevent injury—and adenomatous nodules were produced.

The working hypothesis, therefore, had a basis founded on experimental facts. Cells had been made to proliferate both in vitro and in vivo by means of auxetics. Healing of a damaged site presumably is brought about by auxetics set free by the injurious agent, the amount of proliferation being in ratio to the amount of damage, a theory which seems to be a reasonable one.

Some preliminary experiments were then made with soot and with gasworks and blast-furnace pitch. Using living human lymphocytes as the test cells and employing the jelly method, it was possible to detect auxetics in the soot and gasworks pitch, but only to a very slight degree, if at all, in most varieties of blast-furnace pitch. Later on, H. Bayon succeeded in producing a condition resembling the early stages of squamous epithelioma in rabbits' ears by injecting watery extracts of gasworks tar and pitch. Similar extracts of blast-furnace pitch, on the other hand, used in a similar way, did not produce this result.

The jelly method further showed that most of the alkaloids excited amoeboid movements in leucocytes and some forms of epithelial cells, an observation originally made by Osler, and the term *kinetic* was given to such substances. It was found that many kinetics, although they do not themselves induce cell-division, have a power of augmenting the action of auxetics in doing so. If an auxetic is present, the addition of a kinetic may increase the action of the auxetic as much as five fold, as can be measured by the quantity of auxetic present. Choline and

cadaverine (pentamethylene diamene), products of putrefaction, are powerful augmentors as well as kinetics, acting in this way like the alkaloids.

All the occupational commodities were then tested for auxetics and kinetics, and some of them for augmenting the former. The cells used were human lymphocytes for auxetic and augmentor, and human leucocytes for kinetic action.¹²

SOOT

A 20 per cent watery extract contains both auxetics and kinetics.

BLAST-FURNACE PITCH

A 10 per cent watery extract contained a trace of kinetic only, and auxetic could only be detected if this action were artificially augmented by an alkaloid such as atropine.

GAS-WORKS PITCH

A 10 per cent watery extract contained both auxetics and kinetics.

GAS-TAR

A 10 per cent watery extract contained both auxetics and kinetics.

BLAST-FURNACE TAR

A 10 per cent watery extract contained no auxetic or kinetic.

There is, then, an experimental difference between blast-furnace tar and pitch and the ordinary gas-works varieties, which is in accordance with the clinical evidence. As already stated, there are two explanations for this difference, one being that blast-furnace tar (and therefore the pitch) is chiefly derived from the less bituminous Scottish coal, the other being that it is pro-

¹² A complete description of the experiments together with the technique will be found in the *Brit. Med. Jour.*, 1911, 1, 884, and 1913, 1, 511; and in *Researches into Induced Cell-reproduction and Cancer*, vols. I-III, (London and Philadelphia), 1911 and 1913.

duced at a lower temperature. Samples of coal from various seams were therefore tested. They were sent to the laboratory distinguished by numerals only, the key being kept at the Mines Department of the Home Office until the tests were complete. It was found that coal does not contain nearly so much auxetic or kinetic as do tar, pitch, or soot. High concentrations had to be made in the extracts (ten times higher than tar) before any action could be detected; and the more bituminous coals contained auxetics and kinetics, while the harder varieties contained only traces or none at all. This seems to be the explanation why blast-furnace tar and pitch contain less auxetics and kinetics. From this time onwards all experiments were of the "blind" variety.

In order to control the foregoing experiments, and to ascertain which fractions of tar are responsible for the auxetics and kinetics, a series of experiments was made with thirty-two samples of tar, pitch, and various oil fractions derived from them. The difference between blast-furnace tar and pitch and the gas-works varieties was again detected, and it was demonstrated that the auxetics and kinetics are contained in the middle fractions of tar. The lighter oils, such as the naphthalene and creosote fractions, are free. It was also found that the auxetics in tar become volatile at about 350°C.

These facts correspond with the theory at the works that the mischievous elements are in the anthracene fraction, but since anthracene itself is not an auxetic, it is evident that the auxetics are impurities in that fraction.

Mrs. Norris, working in these laboratories, has recently been able to isolate two auxetics from tar. One is a substance apparently identical with pseudocumidine, which is only a feeble auxetic, the other is an active base, having an empirical formula $C_{22}H_{14}O_9N_6$, but nothing is known as to its constitutional formula.¹³

Hence the auxetic and kinetic content of tar and its derivatives seems to harmonize with the incidence of the disease

¹³ Note on the bases of gasworks coal-tar which are believed to be the pre-disposing cause of pitch cancer with special reference to their action on lymphocytes together with a method for their inactivation. *Biochem. Jour.*, 1914, viii, 253.

caused by these commodities; the more concentrated they become, the more auxetic and kinetic they contain up to a certain point when the auxetics become volatile. Unfortunately this point, 350°C., is too high for a serviceable pitch to be prepared, or the problem at the briquette works would appear to have a simple solution. Such a pitch is too hard and contains too much carbon for it to be used as a binder for briquettes, and its calorific value is greatly reduced. The auxetics, being soluble, can be washed from the tar, but this also was objected to by the trade because of difficulty in removing water from tar. Its presence causes deterioration of the pitch and danger from explosion during distillation.

OTHER TAR PRODUCTS

Certain tar antiseptics contain auxetics, and some of the capsules advertised for internal administration also have this action.

Naphthalene, which is converted into the harmless shoe-blackening, has not this action.

PETROLEUM

Some samples of commercial refined paraffin oil (kerosene) contain auxetics, and vaseline also has this action. A series of "interim oil scales" and heavy lubricating oil fractions from Oakbank, Addiwell, and Broxburn, in Scotland have been tested. None of them apparently contain auxetics, but four out of seven contain kinetics, and three out of the four kinetic samples are also augmentors.

TOBACCO

Tobacco contains auxetics. Ten per cent watery extracts of ordinary pipe tobacco showed this action to a considerable degree, many cells exhibiting division figures when the 10 cc. of jelly contained 1 cc. of the extract. The water in a "hubble bubble" pipe, through which tobacco smoke had been drawn from 50 grams of several forms of prepared tobacco, also gave an auxetic reaction, but a watery extract of a cigar did not appear so active, it requiring a greater strength of extract.

ARSENIC

Soon after kinetic action was first observed with the jelly method, it was found that arsenic salts in some strengths excited amoeboid movements in leucocytes, and Pye-Smith's recent investigations have prompted more experiments with them. Like the heavy petroleum fractions, certain arsenic compounds are not auxetics, but some of them are augmentors when added to organic auxetics. Arsenic oxide, salvarsan, atoxyl, and sodium arseniate are all kinetics, the first being the most active, the last the least. When extracts of meat were used as the auxetic, the first three compounds proved to be augmentors in the order named as regards strength, but the sodium salt was negative. The jelly on which the cells were spread required at least 3 cc. of a 1 per cent solution of the arsenic compound to produce this augmenting action. But no such action occurred with an artificial auxetic such as theobromine.

By itself, then, arsenic does not appear to produce cell proliferation, but in the presence of products of cell death—such as the extract of meat used in the experiments—it augments the action of the auxetics contained therein. It is possible that chronic skin eruptions due to arsenic are caused by this action. The oxide is the most active salt *in vitro*, but there is no evidence as to the combination in which the drug is held in the living tissues.

ANILINE DYES

Many of these, especially methylene blue, azure I and II, eosin, neutral red, etc, have an auxetic action on blood cells *in vitro*, and when injected into tissues *in vivo* also produce cell proliferation. In view of the fact that their chemical constitutions are so different, it is probable that they produce this action by causing cell death in the first instance, the cell death in its turn setting free auxetics. Cells will live with their granules stained, but their death occurs as soon as the intranuclear chromatin is reached by the stain.

MANURE, PUTRID WOOD CHARCOAL, PRODUCTS OF SUPPURATION
IN ULCERS

These all come into one category. Auxetics can be detected in decaying organic matter, and, if the putrefaction has progressed, kinetics with augmentation are also present. Drew has recently tested pond water for these actions, and has found them increased after the cell death of the winter, suggesting that they are responsible for the awakening of pond life in the spring. There seems to be a relationship between the content of auxetics and albuminoid ammonia. It was with a putrid extract of a dead tissue that cell division was first induced in lymphocytes in 1909. But if the putrefaction has progressed beyond a certain degree, poisonous substances are produced which cause cell death.

Experimentally, therefore, the action of the commodities can be explained by the auxetics or augmentors contained in the commodities themselves. They become impacted in the tissues, either in the hair follicles or the sebaceous glands, or perhaps in fissures caused by slight mechanical injury. Auxetics are dissolved out of the commodities and set up cell-proliferation, giving rise to thickening and warts. The warts slough and drop off, leaving an open ulcer, in which the over-prolific cells at the edge are prone to become malignant. The malignant invasion, judging by clinical evidence, seems to be due to some additional factor, possibly connected with production of more kinetics, which excite amoeboid movement and still further augment the proliferation. From the clinical evidence in occupational cancer, the additional factor appears to come from without. The apparent necessity for open ulceration, and the fact that it is the dirtier parts of the skin which are more liable to the disease, are points worthy of consideration, although the suggestion is as yet entirely theoretical and there is no absolute proof that it is correct.

Auxetics are produced as age advances, the physiological increase of cell death during senescence having this result. It is part of the working hypothesis that the predisposition to cancer

during senescence may be due to a predisposition to cell proliferation caused by auxetics physiologically free in the tissues in general. And, as already pointed out, so may it be with mechanical and chemical injury; the predisposition to cancer brought about by them may also be due to the auxetics set free locally by the trauma. But in occupational cancer, in addition to the auxetics produced by any mechanical or chemical trauma, there are also those contained in the commodities themselves which would produce, and which actually seem to produce, a greatly increased if not a specific predisposition.

The unsatisfactory part of the above work, however, is that it is based on a working hypothesis. There is always a danger, when working in this way, of being biased in favor of a hypothesis; but in this case there was no alternative, and all the experiments were "blind" ones. Since one cannot distinguish by appearance between varieties of tar, pitch, and oils, the experimenters had no idea which was under investigation at any given time; and it is remarkable how accurately the results of the experiments harmonized with the clinical facts. Still, when elaborating such a hypothesis, even though, as in the case in point, it is based on direct experimental evidence, great care must be exercised to prove each step. In the cancer problem, unfortunately, this is not easy, for it involves so many other problems; e.g., the cause of the reproduction of living matter, of development, metastasis, of death, etc., about which very little is known, so that, at present, as regards occupational cancer, one can only work on the somewhat empirical lines described in this paper.

It should be remembered, therefore, that although there is experimental proof that auxetics stimulate the proliferation of cells, a gap is jumped if we assert that they predispose a tissue to cancer, for there is no definite proof of this. It is true that by auxetics cells may be made to divide when removed from the body; and it is true that auxetics, when artificially inoculated into animals, will produce proliferation amounting to benign tumors, and if continued with augmentation will produce a condition identical in microscopic appearance with epithelioma.

It is also true that benign tumors, in a wide sense, form a predisposing site for cancer, and equally true that the auxetic theory coincides with the clinical evidence. But we cannot assert that this is the way in which the predisposition is caused naturally in the living tissues. Proof cannot be obtained owing to lack of methods. The jelly method enables us to test the action of chemical substances on individual living cells in vitro; Carrel and Burroughs have shown how to grow cells in tissue formation in vitro; and sectioning will show the arrangement of cells after death. But there is no method by which we can see with chemical-detecting eyes, so to speak, into a living tissue in its natural surroundings. Therefore we cannot see whether, or how, auxetics work, except under experimental conditions.

An effort is being made, however, to prove the auxetic theory by destroying those substances in tar on a large scale, and observing whether this destruction prevents the incidence of warts and cancer at the briquette works. Mr. H. W. Robinson, of Messrs. Robinson Brothers, tar distillers, West Bromwich, has suggested that the application of the Sorensen reaction might be practicable. Formaldehyde inactivates amino substances, and tests have proved that it destroys the auxetics in tar. Three years ago this was tried on a considerable scale. Messrs. Bird and Son, Cardiff, prepared a large quantity of pitch made from tar treated with formalin, and this pitch was used exclusively at one briquette mill of the Crown Preserved Fuel Company at Cardiff. It was originally intended for this experiment to continue for six months, but unfortunately, owing to unforeseen circumstances, a mechanical breakdown occurred at the tar-distilling works, and the experiment came to an end in two months. The time was too short for the medical commission, who were nominated by the Home Office, and who had previously examined the men's skin, to offer an opinion on the result. The war then broke out, and the shortage of formaldehyde prevented a resumption of the experiment. But the government has promised to undertake it anew as soon as possible, and it is to be hoped that it will then be carried out on a larger scale, the whole of one briquette works to use nothing

but treated pitch for one year (about 50,000 tons) and the other works to act as controls.

If the destruction of auxetics in tar leads to the prevention of occupational cancer at the briquette works, it would appear that the case against auxetics is established.

POSTSCRIPT

Since this paper was written, the writer has seen an excellent paper by Dr. Walter J. Heiman in the *Journal of Cancer Research*, 1916, i, 343, on "Precancerous dermatoses." It complains that the expression "precancerous" is misleading. True, the term is wrong; as Dr. Heiman points out it implies a constant forerunner of cancer. But the expression is not common in Great Britain, and the writer never heard it mentioned in the United States seven years ago.

As was mentioned in the discussion of chronic irritation, many lesions, whether caused by physical force (mechanical injury) or by chemical action (poison), undoubtedly lead to cancer, some occasionally, some more frequently; but it does not necessarily follow that every mechanical or physical trauma is always followed by cancer. Especially in medicine, one can take stock phrases too literally. "Predisposing" seems to be a better term than "precancerous," implying as it does liability, or a more frequent occurrence than usual.

It seems probable, therefore, that the majority of the dermatoses and conditions to which he refers are probably the result of chronic injury (mechanical or chemical) and might be classified as such; but the more occupational predisposition, as already shown, falls into a different category from a clinical point of view, in that a malignant outcome is more constant and the commodities themselves contain or produce a definite predisposing agent.

Kangri cancer is not due to burns only, as Dr. Heiman assumes, but also to the remarkable form of soot which is rubbed into the burn. It is not a little dangerous for Dr. Heiman to draw deductions from his thirteen cases, for the Pearson-Poisson

formula would show a large error of random sampling. But when he states that "we lack a control, a standard of comparison" he strikes a true note, which the writer thinks applies to many issues in cancer research.

REFERENCES

- (1) LUSH, A.: First and second reports on draft regulations proposed for the manufacture of patent fuel. H. M. Stationery Office.
- (2) LEGGE, T. M.: Special report on ulceration of the skin and epitheliomatous cancer in the manufacture of patent fuel. Home Office.
- (3) PYE-SMITH: Arsenic cancer. *Lancet*, 1913, i, 1313.
- (4) CROPPER AND DREW: Researches into induced cell reproduction in amoebae, London, 1911, and 1913.
- (5) ROSS, H. C. AND CROPPER, J. W.: Gasworks pitch industries and cancer. *Brit. Med. Jour.*, 1911, i, 884; and 1913, i, 511.
Gasworks pitch industries and cancer, London, 1913.
- (6) THORNTON, H. G., AND SMITH, G.: *Proc. Roy. Soc., Series B*, London, 1915, lxxxviii, 151.

MULTIPLE SKIN METASTASES FROM CANCER OF INTERNAL ORGANS¹

NOBUYOSHI SUZUKI

From the Institute of Pathology and the Medical Division of Kyoto Imperial University Medical School, Japan

Received for publication, December 6, 1917

INTRODUCTION

In cancer of the internal organs, metastasis into the skin is so rare that Gurlt observed not a single case among 16,637 patients, and neither Reichelmann (711 cases), nor Gussenbauer and Winiwarter (903 cases of gastric cancer), mentioned the occurrence of secondary tumors in the skin. Redlich, Heimann, and Krasting, however, recorded 2 cases out of 496, 2 out of 20,000, and 2 out of 12,730 patients respectively, Lex 7 out of 1183, and Mielecki 3 out of 487 cases. Daus collected 38, Sudo 72, and Kaufmann and Wolf 78 cases from the literature and from among their own patients.

The present paper is an analysis of 110 cases which the writer has collected from the literature, to which are added 5 cases that have come under his own observation. One of the 5 was very kindly brought to his attention by Dr. Hojo.

THE AUTHOR'S OWN CASES

The fragments examined were taken from various organs which had been preserved in alcohol after formalin fixation, and stained with hematoxylin and eosin, Van Giesen's stain, or when necessary, by Mallory's, Bielshowsky's, or Weigert's (elastic fiber) method.

¹ The author has not read the proof of this article.

Case I. M. M., farmer, forty-six years old

Status præsens. There is a tumor on the right side of the neck about half the diameter of the palm, and multiple nodules in the integument of the chest and back; these are of various sizes, bluish, indistinctly defined, flat, firm, painless, and freely movable. The cervical and submaxillary lymph-nodes are not palpable, but those of both inguinal regions and both axillae, particularly the latter, are enlarged and firm. The entire body, especially the lower limbs, is very edematous. There are no other findings of importance.

Autopsy findings. Well nourished male of large frame; general edema.

Skin. There is a hard flat tumor of irregular and indistinct outline, about half a hand's breadth in diameter, on the right side of the neck. In the integument overlying the front and lateral regions of the chest and the upper half of the back, there are nodules varying in size from a hazel-nut to a child's palm; in the epigastric region there is one about the size of a small peach, while a little below the right costal arch there lies still another, this one about the diameter of a hazel-nut, flat, irregular in shape and indistinctly defined.

Many lymph-nodes in both axillae and both inguinal regions (especially on the left side) are enlarged, varying from the size of a bean to that of a sparrow's egg. A number of the bronchial nodes are equally involved, but those of the cervical and supraclavicular regions are not so much affected.

Suprarenals. Around the left one the connective tissue is greatly increased, and the gland has increased to the size of a hen's egg.

Stomach. At the lesser curvature especially there is a distinct cancerous change and in the middle of this curvature a small shallow ulcer. The cardiac end and the lower part of the esophagus are involved by some slight cancerous infiltration, but in the pylorus and the other parts there is no pathological change worthy of note.

Duodenum and small intestine. Negative.

Large intestine. There is a hard tumor about the size of a bean in the upper part of the sigmoid flexure.

Mesentery. Many mesenteric lymph-nodes are swollen to the size of a hazel-nut or a pea. In the middle of the small intestine there is a hard thickening 3 cm. in diameter, nearly round in shape, with a center about two finger-breadths from the intestinal canal. Its margin is formed by fat, and the thickening itself presents a radiating structure like a chrysanthemum.

Retroperitoneal lymph-nodes. Not greatly enlarged.

Microscopical examination. Stomach. Carcinomatous ulcer of the lesser curvature, invading the muscular layer, the subserous connective tissue, the lymph-channels, and the blood-vessels.

Skin. The epithelium is normal. In the corium and the subcutaneous stratum the connective tissue is greatly increased, and cancer cells are embedded in it singly or in groups. Hair-follicles, sebaceous glands, and the region around them show no pathological change, but cancer cells are to be seen about the sweat glands, some of which show traces of atrophy. There are cancer cells in the lymph-vessels here and there, but none are visible in the blood-vessels. The corium and the subcutaneous layer show a great increase of the lattice-fibers in addition to a distinct increase of the collagenous fibrils, especially around the sweat glands. Where the cancer cells lie in groups, the lattice-fibers surround every cancer cell like the strands of a net.

Suprarenals. Cancerous infiltration.

Lymph-nodes. Mesenteric, retroperitoneal, bronchial, both axillary, cervical, inguinal, and supraclavicular groups infiltrated by cancer cells; increase of connective tissue, and infiltration by eosinophiles.

In the blood-capillaries and small veins of the medulla of the left kidney and the submucous stratum of the bladder, and in the small arteries of the lung, emboli of cancer cells.

The cells of the metastatic growths have undergone mucous degeneration and contain small vacuoles. On account of these vacuoles, the cells are enlarged, their nuclei being pressed to the margin making them look like signet-rings. The cells in the chrysanthemum-shaped thickening in the mesentery contain particularly large nuclei (giant nuclei), some of them four or five times as large as those in the other metastatic growths.

Case II. N. N., forty-three years old

Status præsens. The skin and the mucous membranes are icteric. On the front and lateral parts of the chest and abdominal wall there are innumerable small hard tumors varying from the size of a hempseed to that of a pea, distinctly outlined, tender, and freely movable on the underlying tissues. Lymph-nodes not palpable. There is peristaltic unrest of the stomach. In the epigastric region it is possible to feel a resistance, but no tumor.

Autopsy findings. A medium-sized, delicately built, emaciated male.

Skin. Universally icteric. On the front and lateral parts of the trunk, and especially on the abdominal wall, there are innumerable tumors varying from the size of a millet seed to that of a sparrow's egg; these are hard, distinctly outlined, and freely movable.

Heart. Many small hard nodules under and in the serous membrane of both vestibules.

Lungs. Intact. Bronchial lymph-nodes not enlarged.

Great omentum and mesentery. Many small firm tumors in the neighborhood of the lesser and the greater curvatures of the stomach.

Kidneys. Multiple small hard tumors in the fat capsules of both kidneys.

Suprarenals. Both of normal size; a nodule about the size of a pea in the connective tissue around the left one.

Liver. Periportal lymph-nodes enlarged. Metastatic growths in the gall-bladder.

Stomach. The pyloric part contains an annular ulcerated swelling.

In the anterior wall of the pylorus are many minute grayish white nodules.

Duodenum. Mucous membrane 1 cm. from the pyloric ring is firm and uneven.

Small intestine. Grayish white tumor the size of a lentil 50 cm. from its uppermost part. All along the small intestine there are about twelve similar growths, especially numerous at the ileocecal part.

Pancreas. Normal size, but very hard. Hemorrhage into the head of the organ; small tumor at the opposite extremity.

Retroperitoneum. Lymph-nodes enlarged and very hard, with a marrowy appearance on the cut surface. The fat 7 cm. below the ileocecal portion, the great omentum, ileocecal fold, transverse mesocolon, and pancreatico-gastric ligament contain many small firm nodules. Similar growths in the subperitoneal connective tissue of the diaphragm.

Prostate. Somewhat enlarged and shows on section metastatic knots which look marrowlike.

Microscopical examination. Stomach. The tumor at the pyloric end is an adenocarcinoma, which is invading the stomach wall and its small blood-vessels and lymph-channels. The lymph-nodes of the pyloric and cardiac regions are also involved.

Skin. Extension of the secondary growths by way of the lymphatics.

Heart. Metastatic carcinoma.

Lungs. Cancer cell embolus in a small bronchial artery of the right lung. The bronchial lymph-nodes contain metastatic cells only in the marginal lymph-sinus.

Liver. Many cancer cells in the lymph- and blood-capillaries of Glisson's capsule. Metastases in the wall of the gall-bladder, and cancer cell emboli in its smaller arteries.

Pancreas. Metastases only in the connective tissue septa; these contain a few giant cells.

Suprarenals. Metastases in medulla, cortex, and capsule.

Retroperitoneum. The tumors in the fat 7 cm. below the ileocecal region, the great omentum, the transverse mesocolon, the pancreaticogastric ligament, and the subperitoneal connective tissue of the diaphragm, are metastatic adenocarcinomata. They are sharply defined and their cells are closely pressed together into solid masses; the amount of connective tissue is very small.

Prostate. Similar secondary growths.

Kidney. Cancer cells in the blood-capillaries and the small veins of the left kidney.

Retroperitoneal lymph-nodes. Metastatic adenocarcinoma.

Axillary and inguinal lymph-nodes. Not enlarged. Microscopically only a few cancer cells are to be observed in the marginal sinus of some of the inguinal nodes.

Case III. K. K., forty-four years old

Status præsens. Both lateral and posterior cervical lymph-nodes are enlarged. Axillary nodes firm, not tender, and about the size of a pigeon's egg.

In the epigastric region there is a tumor about the size of a hen's egg, which is freely movable and a little tender. In the integument of the abdomen many compact tumors, and they are not tender. They are as large as a pea and situated in the subcutaneous tissue.

Inguinal lymph-nodes. Both sides somewhat enlarged.

Autopsy findings. A middle-sized emaciated female.

Skin. In the subcutaneous tissue of the anterior part of the abdomen there are many firm movable tumors.

Great omentum. In the region of the stomach there is a number of firm grayish white nodules.

Peritoneum. Small soft white nodules.

Stomach. Form normal and size somewhat small. The serous membrane of the cardia is very uneven, and adherent to the diaphragm. The greater curvature is partially adherent to the transverse colon, and the pylorus and the lesser curvature to the pancreas and the retro-

peritoneal and periportal connective tissues. Over all the mucous membrane there are many small, flat, grayish white growths. The whole wall is thickened unevenly, and at the cardia it is especially annular, with its lumen so narrowed as to allow only rice-grains to pass through. In the pyloric region there are no localized pathological changes. The lower part of the esophagus and the duodenum near the pylorus contain small tumors.

Pancre . A number of growths, some as large as a pigeon's egg. Mesenteric, retroperitoneal, inguinal, and left axillary lymph-nodes enlarged.

Small intestine. There are from the beginning to the ileocecal region about 20 small round or elliptical submucous tumors, grayish white in color. In that part of it which is close to the ileocecal valve there is a transverse ulcer 2.0 to 3.5 cm. in width, with margins thickened like banks, and its ground is grayish white and shows many miliary growths. At the beginning of the cecum there are two small tumors.

Kidneys. Some small nodules are localized in the adipose tissue at the hilum of the left one.

Ovaries. In the left one, there are many small grayish white tumors. The right one consists of many rough knots.

Microscopical examination. **Stomach.** The wall of the cardia is very much thickened by an abundant increase of polygonal cells and connective tissue. Mostly adenocarcinoma, but some scirrhous. Muscular coat notably thickened. Between muscle bundles, cancer cells lie singly or in groups.

Skin. The metastatic tumors are situated particularly in the subcutaneous connective tissue, and are not sharply defined, so the cancer cells infiltrate into the connective tissue around the metastatic nodules and through the spaces between the fat cells or the fibers of connective tissue. The cancer cells are not arranged adenomatously, but singly or in groups. Some have swollen mucus thereby becoming "signet-ring cells." In certain parts the connective tissues are increased abundantly, and there is a scirrhous appearance here and there. In these metastases a number of giant-cells and cancer cells with the giant nuclei are to be found. The cancer cells also form metastases in both ovaries, small intestine (mucous and submucous connective tissue), mesentery, great omentum, and in the periportal, inguinal, axillary, retroperitoneal, and mesenteric lymph-nodes. The cancer cells are arranged adenomatously, singly, or in solid masses.

Case IV. H. O., sixty-one years old

Status præsens. Abdomen. Very much distended; the contour of the stomach is distinctly visible. At times a fist-sized, hard, contracted intestinal knot and a peristaltic movement are visible in the abdomen. The abdomen is tender all over when pressed, but there is no particular seat of pain, nor any hard circumscribed tumor palpable; on percussion somewhat tympanitic. No fluctuation is observed.

Autopsy findings. A middle-sized, large framed, emaciated male.

Skin. In the integument of the epigastrium there is a fresh wound, made by the extirpation of a metastatic tumor. In the subcutaneous tissue of the sacral region there is a tumor about the size of a large cherry.

Thorax. No mediastinal lymph-nodes are enlarged.

Liver. On the surface and on section there are many firm tumors, some as large as a pecan nut. A periportal lymph-node is enlarged to the size of a bean.

Rectum. Annular tumor the size of an egg, with ulcerated and uneven surface, about 4.5 cm. above the anus. By this tumor the lumen of the rectal canal is narrowed. At the upper part of the rectum, namely, the part corresponding in location to the bottom of the pelvic cavity, there is a perforation, large enough to allow the thumb to pass through. Other abdominal organs show no pathological change.

Lymph-nodes. Retroperitoneal, left supraclavicular, and both iliac-nodes, bean-sized, enlarged and somewhat hard.

Skull. Parts of the left temporal bone and of the sphenoid bone are softened. So the bone substance in these parts became lost, and there is a soft tissue in these places.

Microscopical examination. The tumor is an adenocarcinoma which has invaded the wall of the rectum.

Liver. The metastatic growths are generally adenocarcinoma. The cells are arranged in adenomatous structure, but at some parts are pressed closely together into solid masses; there is no increase of connective tissue.

Skin. The metastatic knots are situated in the subcutaneous tissue, and are not sharply defined. The cancer cells are arranged in adenomatous form but some groups have no lumen. There is a well developed stroma between these cancer cell masses.

Sphenoid bone. Metastases.

Retroperitoneal, periportal, and iliac lymph-nodes:—Metastases.

Case V. (Courtesy of Dr. Hojo.) T. Y., forty-three years old

Status præsens. The patient is a very emaciated man. In the stomach region a resistance is felt on palpation, and the patient feels a dull pain when the stomach region is pressed. The cervical, axillary, and inguinal lymph-nodes are enlarged from sparrow's to hen's egg size.

Subcutaneous tumors, varying in size from that of a bean to that of a hen's egg, are found scattered over the abdomen, shoulder and back, right thigh (internal aspect), lower part of leg (anterior aspect), and the sacral region.

Microscopical examination. (Nodules in the integument of the thorax and the thigh.) The tumors are located mostly in the subcutis, but the cancer cells have infiltrated continually into the corium. These cells are all polygonal, and arranged mostly in one layer, but some in many layers, so that they form narrow irregular lumina. Their nuclei and protoplasm can be stained distinctly. They seem to have no tunica propria.

Thus we were led to think that these were skin metastases of the adenocarcinoma and from the clinical symptoms the primary organ was perhaps the stomach.

SUMMARY OF PRECEDING FIVE CASES

To sum up these 5 cases of the skin metastases of the cancer:

a. Sex. Four of the cases were male, 1 being female.

b. Age. Four were between forty and fifty years of age and 1 between sixty and seventy.

c. Organs primarily affected (autopsy made in 4 cases). The stomach in 3 cases, and the rectum in 1 case. The other remaining case was perhaps the stomach.

d. Localization and structure of the skin-metastases.

Case I. The neck, the thorax, the back, and the abdomen.

Case II. The thorax and the abdomen.

Case III. The abdomen.

Case IV. The thorax and the sacral region.

Case V. The abdomen, the back, the shoulder region, the leg, the sacral region, and both inguinal regions.

As to structure and position in the skin, in 4 cases the metastases were tuberous and localized mostly in the subcutaneous

tissue while in 1 case there were diffuse flat eminences in the corium and the subcutaneous connective tissue. Four cases were adenocarcinoma, and 1 scirrhus. Of the 4 cases of adenocarcinoma, 1 was sharply defined with no increase of the connective tissue, while the other 3 had some increase of the connective tissue and were somewhat indistinctly defined; the third case showed scirrhus here and there and in this case cancer cell masses were mixed with some giant cells with giant nuclei.

e. Type of metastasis.

Case I. We can not exclude hematogenous metastasis as there were cancer cells in the blood-vessels of the lungs, kidneys, and the submucous stratum of the bladder. But we may assume that the skin metastases were probably lymphogenous, because lymph-capillaries and spaces in the skin were dilated here and there, and some of these capillaries and spaces contained the cancer cells, and around some sweat glands cancer cell infiltration was especially remarkable. Again, surrounding all the skin glands lay a net of lymph-vessels, narrowed at the points where they intersected (Ribbert); therefore it seems to be easy to form metastases at this place; and the regional lymph-nodes showed carcinomatous metastases and were greatly enlarged.

Case II. Owing to the following four factors, the skin metastases were hematogenous:

The nodules all appeared simultaneously.

Little or no involvement of the regional lymph-nodes was observed.

No dilatation of the lymph-vessels and spaces in the skin was seen.

The existence of cancer cells in the blood-vessels and capillaries in the metastatic growths, and also the presence of cancer cells in the blood-vessels of the lungs.

Case III. In this case the metastases were formed perhaps in the lymphatic way, owing to the following four factors: The localization of the skin metastases was only in the abdomen, and not scattered in distant places. The non-existence of cancer cells in the blood-vessels in the metastatic growths.

No existence of the metastases in the lungs and liver.

The distinct cancerous change of the regional lymph-nodes.

Case IV. The skin tumors in this case were probably hematogenous, owing to the next two factors:

The existence of the metastases in the liver and bones.

The skin metastases existed in two distant places, namely, in the chest wall and sacral region.

f. In 3 cases the patients died within about four to six weeks from the time of the first appearance of the skin-metastases while in 2 other cases the time is not exactly known.

g. Other notes.

Case I. The metastatic places were especially the connective tissues, and there the connective tissues were increased greatly.

In the growths existed abundant small round-cell infiltration, and abundant eosinophile cells were mixed with it.

There were metastases in the sympathetic ganglia in the neighborhood of the left suprarenal, so some ganglion cells showed tigrolysis and pycnosis.

Case II. Although the metastatic places were particularly the connective tissues as in the first case, this case showed no increase of the connective tissue.

The giant cells existed in the metastatic growth in the pancreas.

STATISTICAL OBSERVATIONS

The cases upon which the following conclusions are based will be found in tabular form at the end of the article.

(1) Sex. In regard to the sex I have obtained the following result:

	NUMBER OF CASES		CASES IN FOREIGN COUNTRIES		CASES IN JAPAN	
		<i>per cent</i>		<i>per cent</i>		<i>per cent</i>
Male.....	43	46.7	34	42.0	9	81.8
Female.....	49	53.3	47	58.0	2	18.2

According to the statistics of cancer in general, the number of the female surpasses that of the male in foreign countries, while in Japan there is a great preponderance on the side of the

male; therefore it is not to be wondered at that in the statistics of the skin metastases we find the same preponderance in the cases of female in foreign countries and of male in Japan. It should be stated that in Japan, in the primary cancer of the internal organs, there are twice as many male as female.

AUTHOR	MALE CASES		FEMALE CASES	
		<i>per cent</i>		<i>per cent</i>
Aschoff.....	1506	43.5	1956	56.5
Ishibashi and Takatsu	522	70.3	221	29.7

(2) Age. The age is mentioned in only 79 cases.

AGE	NUMBER OF CASES	PER CENT
Under 10	1	1.3
11-20	2	2.5
21-30	6	7.6
31-40	14	17.7
41-50	25	31.6
51-60	15	19.0
61-70	11	14.0
Over 70	5	6.3
Total.....	79	100.0

The greatest number of cases is between forty and fifty years of age, i.e., about one-third of the total. According to Paul Horn, the great majority of the acute carcinosis also is to be seen in the age between thirty-one and fifty.

(3) Organ primarily affected. In the following 97 cases of skin metastasis the primarily affected organs are accurately diagnosed and mentioned.

The following table shows that the primary tumors are located in more than one half of the cases in the digestive organs. Of these organs, the stomach is most frequently affected; next come the uterus and the intestines, then the lungs, ovaries, etc. Riechelmann and others have demonstrated that in cases of cancer of the stomach and the esophagus, the skin metastases are comparatively few, and those of the liver, the lungs, and the ovaries rather numerous, while this was just contrary in my cases.

PRIMARY ORGAN	CASES OF SKIN METASTASIS		CANCER OF INTERNAL ORGANS (RIEHELMMANN)	
	Number of cases	Per cent	Number of cases	Per cent
Stomach.....	33	34.0	288	48.8
Intestine.....	12	12.4	59	10.0
Esophagus.....	4	4.1	77	13.1
Pharynx.....	2	2.1	4	0.7
Liver.....	4	4.1	3	0.5
Pancreas.....	2	2.1	19	3.2
Uterus.....	15	15.5	86	14.6
Ovaries.....	5	5.2	14	2.4
Kidneys.....	2	2.1	2	0.3
Suprarenal.....	2	2.1		
Bladder.....	1	1.0	6	1.0
Thyroid gland.....	2	2.1	5	0.8
Lungs.....	7	7.2	27	4.6
Bronchus.....	3	3.1		
Trachea.....	1	1.0		
Mediastinum.....	2	2.1		

The following table (from the statistics of Redlich and Feilchenfeld) shows the percentage of metastases in general:

PRIMARY ORGAN	REDLICH	FEILCHENFELD
	<i>per cent</i>	<i>per cent</i>
Mamma.....	100.0	96.2
Pancreas.....	100.0	77.0
Lungs.....	96.6	71.4
Bile-ducts.....	93.7	92.3
Kidneys.....	87.5	
Ovaries, vagina and vulva.....	86.7	
Stomach.....	84.0	85.8
Uterus.....	79.5	59.5
Uterus and vagina.....		
Esophagus.....	75.0	76.8
Pharynx.....	72.7	
Large intestine.....	70.0	53.2
Rectum.....	69.6	66.7

From this table it is apparent that in the case of primary cancer of the internal organs, metastases are most often formed from cancers of the pancreas, bile-ducts, lungs, etc., and less fre-

quently in cancer of the esophagus, pharynx, large intestine, rectum, etc.

Next, the duration of life of the patients must be noted. According to Paul Horn's investigation, cancers of the liver and pancreas progress most rapidly; next those of the lungs and kidneys, as shown in the following tables:

PRIMARY ORGAN	PERCENTAGE OF ACUTE CARCINOSIS. (THE PATIENTS DIE BEFORE THREE MONTHS HAVE ELAPSED)
Liver.....	40.1
Pancreas.....	37.5
Lungs.....	23.7
Kidneys.....	15.4
Suprarenals.....	14.3
Thyroid gland.....	10.2
Small intestine.....	8.0
Stomach.....	3.4

The preceding facts confirm the following statement made by Sudo: Although cancers of the liver and pancreas are more liable to form metastases, there is not sufficient time for the formation of skin metastases because the patients die before the cancer cells can develop in some part of the body distant from the primary organs.

(4) Types of cancers.

NO.	TYPES	NUMBER OF CASES	PER CENT	ACUTE CARCINOSIS (PAUL HORN)
				<i>per cent</i>
1	Adenocarcinoma (medullary cancer).....	27	58.7	84.0
2	Scirrhus.....	11	23.9	11.7
3	Gelatinous cancer.....	3	6.5	4.3
4	Squamous cell cancer.....	3	6.5	
5	Basal cell cancer.....	2	4.4	
	Total.....	46	100.0	100.0

This table shows that adenocarcinoma and medullary cancer most often produce acute carcinosis; next comes scirrhus.

(5) Localization of metastatic tumors in the skin.

The following table mentions only those cases in which the localization of the skin metastases is given in the literature together with my 5 cases.

NO.	LOCALIZATION	NUMBER OF CASES	PER CENT
1	Cutis and subcutis stratum.....	36	55.4
2	Subcutis.....	29	44.6

So I can say that skin metastases develop mostly in the cutis and subcutis simultaneously or in the subcutis only.

(6) Form, number, and size of the metastatic growths in the skin.

The forms of the skin metastases may be classified into circumscribed and diffuse, as follows:

NO.	FORM	NUMBER OF CASES	PER CENT
1	Circumscribed.....	66	90.4
2	Diffuse.....	5	6.9
3	Two forms mixed.....	2	2.7

It is evident from this table that cancer forms metastases in the skin mostly in nodular form, while diffuse infiltration is very rare. I found 3 instances of lymphatic infarction (carcinomatous) in 5 cases of diffuse form, but only 4 cases of it out of 66 cases of nodular form and 1 case of it out of 2 cases of the two forms mixed.

The size of the metastatic tumors in the skin varies according to the growth of the cancer and the duration of the patient's life. In the case of multiple skin metastases, large nodules of different sizes, varying from the size of a pea to that of a walnut, are most numerous, while those of miliary size and those so small as to be hardly perceptible are very scarce; I have found only 4 cases (Röseler, Frank, Regnault, and my case II). Growths as large as a hen's egg are also rare (Sudo, Levi, Reitmann, Clairmont, and my case V).

The diffuse tumors vary in extent and in size. Schlagenhauser's case is probably the most advanced, that is, the skin metastases spread over the front and back neck regions, both supraclavicular fossae, the whole thorax and right abdominal side, backwards to the vertebral column, and the right arm. Next comes Mannoury's case. It was comparatively advanced, having spread over the whole right side of the neck and below the clavicle to the subclavicular triangle. In Geipel's case it was over the whole neck, being particularly stiffly infiltrated on the right side. In Kayser's case it was formed of many different sized metastatic growths, the largest being the size of a palm, located in the left thigh. In Rusch's case it was infiltrated in the supraclavicular fossa. The widest infiltration I have seen was in my case I; it was half as large as a palm and located at the right side of the neck.

(7) Localization of the skin metastases. I have found 81 cases in which the localization of the metastatic growths is mentioned.

LOCALIZATION	NUMBER OF CASES
All over the body.....	10
Thorax.....	37
Abdomen.....	39
Face.....	4
Head (occiput).....	8
Neck.....	11
Nape of neck.....	3
Back, lumbar, and sacral regions.....	15
Arm (hand).....	15
Leg.....	15
Shoulder region.....	5
Inguinal region.....	3
Around the labium.....	1

Thus the abdomen and thorax seem to be the favorite seats of the skin metastases. In other words, the skin metastases are more frequently found on the trunk than in the limbs; more on the abdomen and chest than in the dorsal and lumbar regions. Also when the limbs are affected, it is more on the extensor than on the flexor side.

The localization of the skin metastases is variously accounted for.

1. According to Ribbert, around every gland and hair-follicle in the skin there is a network of intersecting lymph-vessels; therefore, it seems easy to form metastases at these places. This hypothesis may apply to my case I where the cancer cell infiltration around the sweat glands was so extensive. However, I have not found such changes in other cases.

2. In some cases the skin metastases appeared after a herpetic eruption or other skin affection (Schmidt and Mignon), but I found none in any of my cases.

3. Reitmann says that as among stomach cancers forming skin metastases the scirrhus type is quite frequent, the metastases seem to be formed according to the "organ relationship theory" in the similar tissues, viz., in the skin, which has many connective tissues. According to my statistics of stomach cancers with skin metastases, however, there were 8 scirrhus out of 22 (36.4 per cent) and regarding the cancers in all internal organs, there were only 11 cases of scirrhus out of 46 cases (23.9 per cent). Although in some cases many metastatic growths were formed in the skin and other connective tissues, none were formed in organs with few connective tissues (Reitmann, Rösel, Emanuel, Sudo's case III, Kaufmann-Wolf, and my case I). Among these cases, those of Reitmann, Kaufmann-Wolf, and Suzuki were scirrhus. These cases are, however, very rare.

Adenocarcinoma and medullary cancer form skin metastases more frequently than scirrhus. This theory seems to apply therefore only to these few cases.

As to the reason why metastasis takes place on the abdomen and thorax, the following explanations are given.

1. Places full of wrinkles, says Lereboullet, are the most favorable situations of the skin metastases. But the axillary fossa, scrotum, inguinal region, popliteal space, etc., have by far more wrinkles than the abdomen and thorax; yet these localities are not favorite ones for skin metastases.

2. According to Sudo, it is due entirely to the disposition of the places that the thorax and abdomen are the best substratum for the skin metastases.

The favorite situation and the paths of the metastases are not wholly unrelated, so I shall try to make some explanation of this relationship.

There are three modes of forming the skin metastases.

1. The continuous infiltrating growth.
2. The hematogenous metastasis.
3. The lymphogenous metastasis.

I shall now describe in what way the lymphatic metastases reach the skin. In the case of primary cancer of the organs in the thorax, the cancer cells affect the bronchial and tracheal lymph-nodes first, next the cervical, supraclavicular, and axillary nodes. Through these the metastases reach the skin.

The lymph-channels of the lungs, bronchi, lower part of the trachea, and some from the esophagus and the heart flow into the bronchial nodes. The lymphatic stream from the bronchial nodes flows into the bronchomediastinal trunk and vein-angle. The connecting point of the paratracheal and tracheobronchial nodes with supraclavicular is the important flowing way. The lateral group of the supraclavicular nodes connects with the axillary nodes. It is possible that the metastases are formed from these nodes retrograding in the skin of the thorax, shoulder, and arm.

In some cases the metastases are formed from the thoracic organs retrograding into the abdominal organs or their lymphatic nodes.

The primary cancer of the abdominal organs forms metastases in the retroperitoneal lymph-nodes. From these the cancer cells pass through the lymphatic vessels to the cervical, supraclavicular, intraclavicular, and axillary nodes. Thus the metastases are formed in the skin.

In other cases, the metastases are formed in the iliac first; next in the inguinal lymph-nodes, so the cancer cells reach the skin through the *vasa lymphatica superficialia abdominis*.

In some cases, however, the metastases pass from the iliac to the lower epigastric lymph-nodes and on to the navel ring.

There are probably still other ways: namely, from the retroperitoneal nodes through the *vasa lymphatica profunda abdom-*

inis, then through the vasa lymphatica superficialia abdominis to the skin. From the periportal lymphatic nodes to the navel via ligamentum teres hepatis. So it is not infrequently that metastases are formed at the navel.

In the before stated lymphatic paths, the metastases are formed most frequently over the abdomen and thorax. In cases where the limbs are affected, it is quite frequently the flexor side. It is not to be wondered at that this should be so in the case of lymphatic metastases, if we stop to think that the lymphatic system is more developed anatomically on the flexor side of the limbs and on the thorax and abdomen, than on the extensor side or in the dorsal and lumbar regions.

It is very seldom, however, that we find both navel and skin metastases in the same case, and of the 166 cases before mentioned, only 4 were described as navel metastases (Cahn, Spiegelberg, Freund, and Stropeni).

Metastases rarely exist in the regional skin when the inguinal, cervical, and axillary nodes are distinctly swollen. Although these lymph-nodes are greatly enlarged and metastases exist in the regional skin, we can not easily pronounce it to be lymphogenous metastasis, because it may be possible that, through the skin metastases, the regional nodes are secondarily swollen. It is difficult to decide which were swollen first.

Next I shall describe the blood-vessels. According to the reports of Spalteholz, Lewinski, and others, in the palm of the hand, the sole of the foot, the gluteal and patellar regions, etc., where there is much external pressure, there are many blood-vessels. There are few, however, in the skin of the abdomen and here there are few anastomoses. In the back there are more blood-vessels and more anastomoses. In the limbs, the blood-vessels are generally more numerous on the flexor than on the extensor side. Therefore the skin metastases are formed apparently more on the side where there are few blood-vessels.

Accordingly, the question why the metastases of the cancer cells on the skin of the thorax and abdomen are of frequent occurrence, is difficult to answer. A careful consideration of the anatomical distribution and condition of the blood- and

lymph-vessels in these regions may throw light to some though not to sufficient extent. I support Sudo in the statement that it is due to the disposition of the place that the abdomen and thorax are the favorite situations of the skin metastases.

(8) Clinical observations.

Clinical diagnosis of skin metastases is sometimes very difficult, especially when the symptoms of the primary cancer do not manifest themselves (Lippmann, Vierth, Beitzke, Stropeni, Clairmont, Sudo, etc.). In such cases it is necessary to differentiate them from sarcomatosis cutis, multiple fibromata, metastatic endothelioma (Riehl's case), mycosis fungoides, leukemia, etc.

There are some cases which have been diagnosed as other tumors; namely, Göbel's case as fibrolipoma, Sudo's case as multiple fibroma, Reitmann's, Joseph's, and Sudo's cases as sarcomatosis cutis. It is decided in most cases by the histological observation, but there are some cases which have histological representations which increase the difficulty of decision (Reitmann). When the skin tumor is microscopically the adenocarcinoma, there is no doubt that it is mostly cancer of the digestive organs, though there is no pathological symptom ((Ziegler, Stropeni, and Sudo). Especially when it has any liver cells, we can at once diagnose it as the cancer of the liver (Clairmont). When the skin metastases appear, the prognosis is naturally absolutely bad, as there is no good treatment for it. I have made investigations concerning the duration of life from the appearance of the skin tumor to the death of the patients as follows:

TIME BETWEEN THE FIRST APPEARANCE OF SKIN METASTASES AND DEATH OF PATIENT	NUMBER OF CASES
Within a month.....	12
One to three months.....	10
Three to six months.....	12
One-half to one year.....	2
More than one year.....	2

Thus I can say that most of the patients die within three months from the first appearance of the skin metastases.

SUMMARY

1. As to the sex of the patients observed in Japan, out of 11 cases, 9 were male, including the writer's cases.
2. Skin metastasis usually occurs between the ages of forty to fifty.
3. In the majority of cases it has its origin in the digestive organs, which are undoubtedly the most favorite seats of the tumor.
4. In most instances the tumor is adenocarcinoma and medullary cancer.
5. The skin metastases develop mostly in both the cutis and subcutis simultaneously or in the subcutis alone.
6. The circumscribed nodules, pea to walnut in size, are the most frequent, while the infiltrating form is exceedingly rare.
7. The cutaneous surfaces of the thorax and abdomen are the favorite seats of the metastases.
8. Occasionally it is difficult to diagnose the lesion without histological examination.
9. The prognosis is always grave, most cases terminating fata y within three months from the time of the first appearance of the skin metastases.

TABLES

NO.	AUTHOR	SEX	AGE	SEAT OF PRIMARY TUMOR	HISTOLOGICAL TYPE	LOCALIZATION OF SKIN METASTASES	LOCALIZATION OF TUMOR IN SKIN	FORM OF METASTATIC SKIN TUMOR	TIME BETWEEN FIRST APPEARANCE OF SKIN METASTASES AND DEATH
1	Sudo	F	42	Stomach	Medullary cancer	Face, neck, thorax, abdomen, shoulder, and left arm	Cutis and subcutis	Nodular	6 weeks
2	Sudo	M	56	Stomach?	Adenocarcinoma	Thorax, abdomen, shoulder, back, and both thighs	Subcutis	Nodular	5 months
3	Sudo	M	30	Stomach	Adenocarcinoma	Whole body, especially thorax and abdomen	Cutis and subcutis	Nodular	5 months
4	Sudo	M	65	?	Adenocarcinoma	Back and right thigh		Nodular	
5	Arning	M	40	Stomach?	Adenocarcinoma	Whole body	Subcutis	Nodular	
6	Lippmann	M	67	Stomach		Thorax, abdomen, arm above elbow, and both thighs	Cutis and subcutis	Nodular	3 weeks
7	Regnault	F	67	Stomach			Subcutis	Nodular	5 months
8	Joseph	M	32	Stomach	Scirrhus	Whole body	Subcutis and cutis	Nodular	5 months
9	Babes and Stolesco	M	45	Stomach	Adenocarcinoma	Whole body	Subcutis	Nodular	3 months
10	Röseler	F	47	Stomach		Whole body, especially abdomen	Cutis and subcutis	Nodular	7 months
11	Daus	F	77	Stomach	Scirrhus	Whole body, especially thorax, abdomen, neck, and thigh	Subcutis	Nodular	

12	Daus	F	48	Stomach	Scirrhous	Front part of thorax	Subcutis	Nodular	5 months
13	Reitmann	F	33	Stomach	Scirrhous	Especially abdomen and thorax	Cutis and subcutis	Nodular	and 2 weeks
14	Beitzke			Stomach	Medullary cancer		Subcutis	Nodular	
15	Oguro	M	44	Stomach	Scirrhous	Head, face, neck, thorax, abdomen, and back	Cutis and subcutis	Nodular	2 months
16	Kreibich	F	65	Stomach	Scirrhous	Thorax, abdomen, and neck	Cutis and subcutis	Nodular	1 year and 6 months
17	Ziegler			Stomach	Adenocarcinoma	Left arm, especially	Cutis and subcutis	Nodular (Lymphatic infarction)	8 months
18	Vierth	M	42	Stomach	Adenocarcinoma		Subcutis	Nodular	About 4 months
19	Merklen	F	68	Stomach		Thorax and abdomen	Cutis and subcutis	Nodular	
20	Brieger	F	44	Stomach		Thorax	Subcutis	Nodular	2 weeks
21	Cited by Krast- ing			Stomach					
22	Cited by Krast- ing			Stomach					
23	Mielecki			Stomach					
24	Lex			Stomach					
25	Lex			Stomach					
26	Nishida	M	67	Stomach?		Thorax and inguinal region	Subcutis	Nodular	1 month
27	Boinet and Olmar	M	53	Stomach		Thorax			

NO.	AUTHOR	SEX	AGE	SEAT OF PRIMARY TUMOR	HISTOLOGICAL TYPE	LOCALIZATION OF SKIN METASTASES	LOCALIZATION OF TUMOR IN SKIN	FORM OF METASTATIC SKIN TUMOR	TIME BETWEEN FIRST APPEARANCE OF SKIN METASTASES AND DEATH
28	Mannoury	F	41	Stomach	Scirrhous	Neck	Cutis and subcutis	Diffuse infil- tration	3 weeks
29	Tilling	M	42	Stomach	Adenocarci- noma	Abdomen and left hy- pochondrium	Cutis and subcutis	Nodular	1 month
30	Vidal	M	50	Stomach		Epigastrium		Nodular	1 week
31	Lionville	M	58	Stomach			Cutis and subcutis	Nodular	2 weeks
32	Dusaussay	M	43	Stomach		Region of the left vii and xi ribs	Subcutis	Nodular	
33	Milner	M	60	Stomach?		Abdomen			
34	Schlagenhafer	F	20	Stomach?	Mucous can- cer	Neck, thorax, abdo- men, back and right arm	Cutis and subcutis	Diffuse infil- tration (Lymphatic infarc- tion)	
35	Moutier and Marre	F	31	Stomach		Subclavicular region, mammary, and left arm	Cutis and subcutis	Nodular (Lymphatic infarc- tion)	2 weeks
36	Geipel	M	38	Stomach	Adenocarci- noma	Neck, shoulder, and right arm (upper part)	Cutis and subcutis	Diffuse infil- tration (Lymphatic infarc- tion)	3 weeks
37	Rusch	M	38	Stomach?		Supraclavicular fossa	Cutis and subcutis	Diffuse infil- tration (Lymphatic infarc- tion)	

NO.	AUTHOR	SEX	AGE	SEAT OF PRIMARY TUMOR	HISTOLOGICAL TYPE	LOCALIZATION OF SKIN METASTASES	LOCALIZATION OF TUMOR IN SKIN	FORM OF METASTATIC SKIN TUMOR	TIME BETWEEN FIRST APPEARANCE OF SKIN METASTASES AND DEATH
53	Charcot			Large intestine					
54	Mielecki			Intestine					
55	Mielecki			Intestine					
56	Lustgarten	M	50	Rectum		Whole body	Subcutis	Nodular	
57	Quenu-Hartmann			Rectum		Left shoulder	Cutis and subcutis	Nodular	
58	Lereboullet	M	71	Rectum	Adenocarcinoma	Thorax and abdomen	Subcutis	Nodular	1 month
59	Simon	F		Rectum	Squamous cell carcinoma				
60	Nobl	M	40	Rectum		Head, trunk, and supraclavicular fossa		Nodular	
61	Landois	M	56	Rectum	Adenocarcinoma	Occiput		Nodular	
62	Redlich	F		Large intestine or ovary		Trunk			
63	Suzuki	M	61	Rectum	Adenocarcinoma	Thorax and sacral region	Subcutis	Nodular	About 4 weeks
64	Babes and Stoicesco	F	42	Uterus	Adenocarcinoma	Whole body, especially lumbar region, neck, and arm (upper part)	Subcutis	Nodular	
65	Lorrain	F		Uterus					
66	Lex	F		Uterus					
67	Lex	F		Uterus					

	Virchow	F	Uterus		Trunk and left thigh	Cutis and subcutis	Nodular and diffuse infiltration	2 years
68								
69	Petersen	F	50	Uterus	Matrix (basal cell) carcinoma	Thigh and around the labium	Cutis and subcutis	
70	Dybowski	F		Uterus		Leg	Cutis and subcutis	
71	Clairmont	F	50	Uterus	Basal cell carcinoma			
72	Clairmont	F	50	Uterus	Carcinoid	Left thigh		
73	Blau	F		Uterus		Below right mamma		
74	Blau	F		Uterus		Thorax and abdomen		
75	Elschnig	F	73	Uterus			Cutis and subcutis	Nodular (Lymphatic infarction)
76	Joseph	F		Uterus			Cutis and subcutis	
77	Kroemer	F	55	Uterus				
78	Buday	F		Uterus		Abdomen (cicatrix of operation and other parts)	Subcutis	Nodular
79	Emanuel	F	60	Ovary	Adenocarcinoma	Abdomen		
80	Briegelberg	F	19	Ovary		Abdomen and umbilicus		Nodular
81	Spiegelberg	F		Ovary				
82	Frank	F	24	Ovary	Adenocarcinoma	Abdomen (cicatrix of operation and inside border of patella)	Cutis and subcutis	Nodular
83	Freund	F		Ovary or uterus		Abdomen, including umbilicus		Nodular
84	Buday	F		Ovary				

NO.	AUTHOR	SEX	AGE	SEAT OF PRIMARY TUMOR	HISTOLOGICAL TYPE	LOCALIZATION OF SKIN METASTASES	LOCALIZATION OF TUMOR IN SKIN	FORM OF METASTATIC SKIN TUMOR	TIME BETWEEN FIRST APPEARANCE OF SKIN METASTASES AND DEATH
85	Harrison	F	50	Ovary?		Trunk, neck, head, etc.			
86	Lex			Lung		Abdomen			4 months
87	Japha	M	58	Lung			Cutis and subcutis	Nodular	
88	Handford	M	40	Lung		Head, episternal fossa, left arm, left thigh	Subcutis	Nodular	3 months
89	Heimann			Lung					
90	Heimann			Lung					
91	Krasting			Lung					
92	Krasting			Lung					
93	Stropeni	F	35	Bronchus	Adenocarci- noma	Left arm, umbilicus, and Mohrenheim's fossa	Subcutis	Nodular	
94	Redlich			Bronchus		Whole body			
95	Domeney	M	29	Bronchus					
96	Pick			Trachea		Finger			
97	Sanger	M	35	Mediasti- num		Head	Subcutis		3 weeks
98	Schmidt	F	62	Mediasti- num		Neck, thorax (man- na), and left arm	Cutis and subcutis	Nodular	
99	Morreau		3*	Kidney			Subcutis		
100	Winkler	M	45	Kidney	Adenocarci- noma	Neck, back, and palm of hand			
101	Laache	M	63	Kidney and suprarenals		Thorax, abdomen, and right arm	Subcutis	Nodular	

* Months.

102	Chaillous	M	27	Suprenal		Head, face, thorax, abdomen, and limbs	Cutis and subcutis	Nodular	4 months
103	Robert	M	27	Suprenal					
104	Kaufmann Wolf	F	54	Liver	Scirrhus	Thorax and neck	Cutis and subcutis	Nodular (Lymphatic infarction)	About 4 months
105	Clairmont	M	52	Liver	Adenocarcinoma	Occiput	Subcutis	Nodular	
106	Friedreich	F	37	Liver	Scirrhus	Thorax and abdomen	Cutis and subcutis	Nodular	3 months
107	Stahr	F	35	Liver	Medullary cancer	Right middle finger			
108	Dahms	F	69	Pancreas		Whole body	Cutis and subcutis	Nodular	5 months
109	Preti	M	72	Pancreas	Squamous cell carcinoma		Subcutis	Nodular	
110	Fillié	F	60	Thyroid gland	Adenocarcinoma		Cutis and subcutis	Nodular	6 months
111	Winwarter	F	35	Thyroid gland	Medullary cancer				
112	Azúa	M	60	Bladder	Scirrhus	Thorax and abdomen	Cutis and subcutis	Nodular	
113	Hanot	F	22	Between uterus and bladder	Colloid cancer	Epigastric region and thorax	Subcutis	Nodular	
114	Heuss	F	31	?		Right forearm, nose and ear		Nodular	6 months
115	Müller, O.	F	52	?		Thorax and neck	Cutis and subcutis	Nodular	

REFERENCES.

- (1) ARNING: Deutsch. med. Wehnschr., 1912, xxxviii, 1666.
- (2) ASCHOFF: Klin. Jahrbuch, 1902, viii, 337.
- (3) AZÚA DE: Rev. clin. de Madrid, 1909, No. 6; abstr. Monatsch. f. prakt. Dermat., 1909, xlix, 42.
- (4) BABES AND STOICESCO: La progres médical, 1895, viii, 113. Cited by Reitmann.
- (5) BEITZKE: Deutsch. med. Wehnschr., 1904, xxx, 897.
- (6) BARTELS: Das Lymphgefäßsystem, Jena, 1909.
- (7) BLAU: Inaug.-Diss., Berlin, 1870. Cited by Kaufmann-Wolf.
- (8) BOINET AND OLMER: Rev. de méd., 1903, xxiii, 717. Cited by Kaufmann-Wolf.
- (9) BRIEGER: Charité-Ann., 1883, viii, 109.
- (10) BRIEGER: Deutsch. med. Wehnschr., 1902, xxviii, 840.
- (11) BRIEGELBERG: Cited by Sudo.
- (12) BUDAY: Ztschr. f. Krebsforsch., 1908, vi, 26.
- (13) CAHN: Cited by Sudo.
- (14) CHAILLOUS: Bull. Soc. Anat. de Paris, 1897, lxxii, 931. Cited by Reitmann.
- (15) CLAIRMONT: Arch. f. klin. Chir., 1909, lxxxix, 513.
- (16) DANS: Virchows Arch., 1907, exc, 196.
- (17) DAHMS: Inaug.-Diss., Würzburg, 1902. Cited by Kaufmann-Wolf.
- (18) DÖMENY: Cited by Sudo.
- (19) DUSAUSSAY: Bull. Soc. Anat. de Paris, 1876, li, 388. Cited by Kaufmann-Wolf.
- (20) DYBOWSKI: Inaug.-Diss., Berlin, 1880. Cited by Winter: Ztschr. f. Geburtsh. u. Gynäk., 1893, xxvii, 101.
- (21) ELSCHNIG: Wien. klin. Wehnschr., 1898, xi, 102.
- (22) EMMANUEL: Ztschr. f. Geburtsh. u. Gynäk., 1893, xxvii, 62.
- (23) FEILCHENFELD: Inaug.-Diss., Leipzig, 1901. Cited by Redlich.
- (24) FILLÉ: Dermat. Wehnschr., 1914, lviii, 676.
- (25) FRANK: Prag. med. Wehnschr., 1891, xvi, 255.
- (26) FREUND: Beitr. z. Geburtsh. u. Gynäk., 1898, i, 362.
- (27) FRIEDREICH: Virchows Arch., 1866, xxxvi, 465.
- (28) GEIPEL: Arch. f. Dermat. u. Syph., 1911, cvii, 397.
- (29) GÖBELL: Deutsch. Ztschr. f. Chir., 1899, lii, 347.
- (30) GURLT: Arch. f. klin. Chir., 1880, xxv, 421.
- (31) GUSSENBAUER AND WINIWARTER: Arch. f. klin. Chir., 1876, xix, 347.
- (32) HANOT: Bull. Soc. Anat. de Paris, 1873, xlvi, 453. Cited by Kaufmann-Wolf.
- (33) HANDFORD: Pathological Transactions, 1888, xxxix, 48. Cited by Kaufmann-Wolf.
- (34) HARRISON: Med. Gaz., London, 1843, February; abstr. Schmidt's Jahrbücher, 1844, xlii, 75.
- (35) HEIMANN: Arch. f. klin. Chir., 1898, lvii, 911; Arch. f. klin. Chir., 1899, lviii, 31.

- (36) HEUSS: Cor.-Bl. f. Schweizer Ärzte, 1899, p. 18. Cited by Kaufmann-Wolf.
- (37) HORN: Beitr. z. klin. Chir., 1911, lxxii, 532.
- (38) ISHIBASHI AND TAKATSU: Verhandl. d. Japan. path. Gesellsch., 1915, p. 487.
- (39) JAPHA: Inaug.-Diss., Berlin, 1892. Cited by Kaufmann-Wolf.
- (40) JOSEPH: Arch. f. Dermat. u. Syph., 1898, xlv, 177.
- (41) JOSEPH: Atlas d. Histopathol. d. Haut, Berlin, 1899, fig. 28. Cited by Kaufmann-Wolf.
- (42) JUNG: Inaug.-Diss., Tübingen, 1906. Cited by Horn.
- (43) KAUFMANN-WOLF: Arch. f. Dermat. u. Syph., 1913, cxiv, 709.
- (44) KAYSER: Arch. f. Gynäk., 1903, lxxviii, 576.
- (45) KRASTING: Zt.-chr. f. Krebsforsch., 1906, iv, 315.
- (46) KREIBICH: Med. Klin., 1909, v, 1436.
- (47) KROEMER: Arch. f. Gynäk., 1902, lxxv, 665.
- (48) LAACHE: Ztschr. f. klin. Med., 1913, lxxvii, 209.
- (49) LANDOIS: Berl. klin. Wchnschr., 1909, i, 1165.
- (50) LEREBoullet: Bull. Soc. Anat. de Paris, 1899, lxxiv, 542. Cited by Kaufmann-Wolf.
- (51) LEVI: Bull. Soc. Anat. de Paris, 1897, lxxii, 701. Cited by Kaufmann-Wolf.
- (52) LEX: Inaug.-Diss., Heidelberg, 1909.
- (53) LIONVILLE: Bull. Soc. Anat. de Paris, 1874, xlix, 42. Cited by Kaufmann-Wolf.
- (54) LIPPMANN: Ztschr. f. Krebsforsch., 1905, iii, 289.
- (55) LORRAIN: Thèse Caiteau, 1876, p. 717. Cited by Kaufmann-Wolf.
- (56) LUSTGARTEN: Arch. f. Dermat. u. Syph., 1897, iii, 289.
- (57) MANNOURY: Bull. Soc. Anat. de Paris, 1876, l, 734. Cited by Kaufmann-Wolf.
- (58) MERKLEN: Bull. Soc. Anat. de Paris, 1876, li, 457. Cited by Kaufmann-Wolf.
- (59) MILNER: Arch. f. klin. Chir., 1904, lxxiv, 1026.
- (60) MIELECKI: Ztschr. f. Krebsforsch., 1913, xiii, 505.
- (61) MOREAU: Cited by Sudo.
- (62) MOUTIER AND MARRE: Arch. de méd. exper., 1910, xxii, 433.
- (63) MÜLLER: Inaug.-Diss., Bern, 1892. Cited by Kaufmann-Wolf.
- (64) MÜLLER: Dermat. Ztschr., 1908, xv, 565.
- (65) NISHIDA: Jour. Soc. Japanese Military Surgeons, xxxi. Cited by Sudo.
- (66) NOBL: Allg. Wien. med. Ztg., 1912, lxxvii, 16.
- (67) OGURO: Jour. Tokyo Medical Society, 1901, xv, 24.
- (68) PETERSEN: Arch. f. Dermat. u. Syph., 1904, lxx, 313.
- (69) PICK: Cited by Sudo.
- (70) PIGGER: Inaug.-Diss., Göttingen, 1899.
- (71) PRETI: Gazz. med. Ital., No. 21 and 22; abstr. Arch. f. Dermat. u. Syph., 1909, cii, 462.
- (72) QUENIN-HARTMANN: Chirurgie des rectums, 1899, p. 137.
- (73) REDLICH: Ztschr. f. Krebsforsch., 1907, v, 302.
- (74) REGNAULT: Bull. Soc. Anat. de Paris, 1887, lxii, 728. Cited by Kaufmann-Wolf.
- (75) REITMANN: Arch. f. Dermat. u. Syph., 1908, xc, 351.

- (76) RIBBERT: Geschwulstlehre, Bonn, 1914, p. 501.
- (77) RIEHL: Wien. klin. Wehnschr., 1896, xlv, 1057.
- (78) RIECHELMANN: Berl. klin. Wehnschr., 1902, xxxix, 728.
- (79) ROBERT: Cited by Sudo.
- (80) RÖSELER: Virchows Arch., 1879, lxxvii, 372.
- (81) RUSCH: Arch. f. Dermat. u. Syph., 1912, exii, 1.
- (82) SÄNGER: Neurol. Centralbl., 1901, p. 1086.
- (83) SCHLAGENHAUFER: Centralbl. f. allg. Path. u. path. Anat., 1904, xv, 611.
- (84) SCHMIDT: Arch. f. Dermat. u. Syph., 1904, lxx, 321.
- (85) SIMON: Bull. Soc. Anat. de Paris, 1900, lxxix, 211. Cited by Kaufmann-Wolf.
- (86) SPALTEHOLZ: Arch. f. Anat. u. Physiol., 1893, xviii, 1.
- (87) SPIEGELBERG: Monatschr. f. Geburtsh. u. Frauenkr., 1859, xiv, 200.
- (88) STAHR: Inaug.-Diss., München, 1896. Cited by Kaufmann-Wolf.
- (89) STROPENI: Ztschr. f. Krebsforsch., 1910, ix, 1.
- (90) SUDO: Gann, 1913, vii, 33.
- (91) TILLING: Inaug.-Diss., Kiel, 1899.
- (92) VIDAL: Bull. Soc. Anat. de Paris, 1853, xxviii, 91. Cited by Kaufmann-Wolf.
- (93) VIERTH: Beitr. z. path. Anat. u. z. allg. Path., 1895, xviii, 515.
- (94) VIRCHOW: Verhandl. d. Gesellsch. f. Geburtsh., 1858, x, 140. Cited by Kaufmann-Wolf.
- (95) WINKLER: Cited by Sudo.
- (96) WINIWARTER: Cited by Sudo.
- (97) ZIEGLER: Verhandl. d. deutsch. path. Gesellsch., 1902, v, 168. Cited by Kaufmann-Wolf.

INDEX

Adenocarcinoma or adenoma of the liver in a mouse.....	275
Biological treatment of cancer, The.....	303
Bullock, F. D., and Rohdenburg, G. L. A histological study of heterologous tumor grafts.....	31
— ——. Experimental "carcinomata" of animals and their relation to true malignant tumors.....	227
— ——. The influence of heat and radium upon induced immunity against transplanted animal tumors.....	181
Burnett, Theo. C., and Robertson, T. Brailsford. The part played by the hydroxy-benzol radical in the acceleration of the growth of carcinomata by cholesterol and by tethelin.....	75
Cancer, Occupational.....	321
— of internal organs, Multiple skin metastases from.....	357
—, The biological treatment of.....	303
Cancerous diseases in Norway, Researches on the.....	107
Carcinochondrosarcoma of the mouse, Significance of the cartilage in a....	47
Carcinoma, Experimental study of the pathogenesis of	1
Carcinomata, The part played by the hydroxy-benzol radical in the acceleration of the growth of, by cholesterol and by tethelin.....	75
— of animals, Experimental, and their relation to true malignant tumors.....	227
Cartilage, Significance of the, in a carcinochondrosarcoma of the mouse....	47
Cases of typical and atypical lymphosarcoma.....	93
Chick embryo, Growth of tumors in the.....	63
Fichera, G. The biological treatment of cancer.....	303
Fruit fly <i>Drosophila</i> , An hereditary tumor in the.....	279
Gade, F. G. Researches on the cancerous diseases in Norway.....	107
Heat and radium, The influence of, upon induced immunity against transplanted animal tumors.....	181
Hematopoietic system, Some phases of radium action with special reference to the.....	127
Hereditary tumor, An, in the fruit fly <i>Drosophila</i>	279
Histological study of heterologous tumor grafts, A.....	31
Hydroxy-benzol radical, The part played by the, in the acceleration of the growth of carcinomata by cholesterol and by tethelin.....	75
Ichikawa, Koichi, and Yamagiwa, Katsusaburo. Experimental study of the pathogenesis of carcinoma.....	1
Itami, Shigemitsu. Adenocarcinoma or adenoma of the liver in a mouse...	275
Liver in a mouse, Adenocarcinoma or adenoma of the.....	275
Lymphosarcoma, Cases of typical and atypical	93
Mackenzie, George M. Cases of typical and atypical lymphosarcoma.....	93

Malignant tumors in man, Fluctuations in the growth energy of, with especial reference to spontaneous recession.....	193
Millet, John A. P., and Mueller, Theodor. Some phases of radium action with special reference to the hematopoietic system.....	127
Mueller, Theodor, and Millet, John A. P. Some phases of radium action with special reference to the hematopoietic system.....	127
Multiple skin metastases from cancer of internal organs.....	357
Occupational cancer.....	321
Pathogenesis of carcinoma, Experimental study of the.....	1
Radium action, Some phases of, with special reference to the hematopoietic system.....	127
—, heat and, The influence of, upon induced immunity against transplanted animal tumors.....	181
Researches on the cancerous diseases in Norway.....	107
Robertson, T. Brailsford, and Burnett, Theo. C. The part played by the hydroxy-benzol radical in the acceleration of the growth of carcinomata by cholesterol and by tethelin.....	75
Rohdenburg, G. L. Fluctuations in the growth energy of malignant tumors in man, with especial reference to spontaneous recession.....	193
— and Bullock, F. D. A histological study of heterologous tumor grafts.....	31
— — —. Experimental "carcinomata" of animals and their relation to true malignant tumors.....	227
— — —. The influence of heat and radium upon induced immunity against transplanted animal tumors.....	181
Ross, H. C. Occupational cancer.....	321
Spontaneous recession, Fluctuations in the growth energy of malignant tumors in man, with especial reference to.....	193
Stark, Mary B. An hereditary tumor in the fruit fly <i>Drosophila</i>	279
Stevenson, Holland N. Growth of tumors in the chick embryo.....	63
Suzuki, Nobuyoshi. Multiple skin metastases from cancer of internal organs.....	357
Transplanted animal tumors, The influence of heat and radium upon induced immunity against.....	181
True malignant tumors, Experimental "carcinomata" of animals and their relation to.....	227
Tumor, An hereditary in the fruit-fly <i>Drosophila</i>	279
— grafts, A histological study of heterologous.....	31
Tumors, Growth of, in the chick embryo.....	63
Woglom, William H. Significance of the cartilage in a carcinochondrosarcoma of the mouse.....	47
Yamagiwa, Katsusaburo, and Ichikawa, Koichi. Experimental study of the pathogenesis of carcinoma.....	1

THE JOURNAL
OF
CANCER RESEARCH

EDITED BY
WILLIAM H. WOGLOM

ASSOCIATE EDITOR
FREDERICK PRIME

EDITORIAL COMMITTEE
OF
THE AMERICAN ASSOCIATION FOR CANCER RESEARCH

JOSEPH C. BLOODGOOD
Johns Hopkins University

H. GIDEON WELLS
University of Chicago

LEO LOEB
Washington University

JAMES EWING
Cornell University

ERNEST E. TYZZER
Harvard University

VOLUME IV

BALTIMORE, MD.

1919

CONTENTS

NUMBER 1, JANUARY, 1919

Virulence or Adaptation? Wm. H. Woglom.....	1
A Mammary Carcinoma in the Cat. Shigemitsu Itami.....	19
An Investigation of the Power of Mesodermal Derivatives to Immunize Mice against Transplantable Tumors. Shigemitsu Itami.....	23
Proceedings of the American Association for Cancer Research, Eleventh Annual Meeting, March 28, 1918.....	43

NUMBER 2, APRIL, 1919

The Effects of x-ray Irradiation on Living Carcinoma and Sarcoma Cells in Tissue Cultures in Vitro. Noriyoshi Kimura.....	95
Further Investigations on the Origin of Tumors in Mice. IV. The Tumor Incidence in Later Generations of Strains with Observed Tumor Rate. A. E. C. Lathrop and Leo Loeb.....	137
Somatic Mutations as a Factor in the Production of Cancer. A Critical Review of v. Hansemann's Theory of Anaplasia in the Light of Modern Knowledge of Genetics. R. C. Whitman.....	181
The Cost of Cancer in Norway. F. G. Gade.....	203

NUMBER 3, JULY, 1919

Primary Spontaneous Tumors of the Testicle and Seminal Vesicle in Mice and Other Animals. XII. Studies in the Incidence and Inheritability of Spontaneous Tumors in Mice. Maud Slye, Harriet F. Holmes, and H. Gideon Wells.....	207
Cancer in Hainan, China. A Preliminary Statistical Study of 131 Operations with Special Reference to Age Incidence, Anatomical Distribution, and Etiology. Nathaniel Bercovitz.....	229
Mortality Statistics of Cancer among Wage Earners: With Observations on the Comparative Incidence of the Disease in the General Population. Louis I. Dublin.....	235
The Lipoids in Tumors of the Dental Region. Kaethe W. Dewey.....	263
The Size of the Spleen in Immune Mice. Wm. H. Woglom.....	281
Multiple Tumors of the Mouse Mamma: Are they Independent or Metastatic? Albert Fischer.....	325

NUMBER 4, OCTOBER, 1919

Attempts to obtain a Transplantable Tumor in the Higher Species of Animals. F. C. Mann.....	331
A Study of the Chemical Composition of the Blood in Cancer. Ruth C. Theis and William S. Stone.....	349
On Spiroptera Carcinomata and Their Relation to True Malignant Tumors; with some Remarks on the Cancer Age. Johannes Fibiger.....	367

VIRULENCE OR ADAPTATION?¹

WM. H. WOGLOM

*From Columbia University, George Crocker Special Research Fund, F. C. Wood,
Director*

Received for publication, December 22, 1917

REVIEW

Although all observers agree that once a mouse tumor has been transplanted it generally yields an increasing number of daughter tumors upon protracted cultivation, it is not yet known whether this is to be referred to an increase in "virulence" on the part of its cells, or to an augmentation in their power to adapt themselves to each new host successively. The former hypothesis has been supported by Ehrlich (1) and Apolant (2), the latter by Bashford, Murray, Haaland, Bowen, and Cramer (3, 4, 5).

The English investigators urged great caution in comparing the growth of transplanted cancer cells with the cultivation of pathogenic microorganisms, and explained the higher percentage of success and the more rapid growth by an increase in the adaptability of the neoplastic cells to each new environment and an elimination of those elements less able to survive.

They observed frequently a rise of the transplantation outcome to a maximum, and a subsequent rapid diminution in the percentage of success, which in turn, was succeeded by an increase to the maximum. By choosing a suitable interval for the inoculation of tumors selected from series with from 90 to 100 per cent of takes, and especially by increasing the dose, the authors had been able to evade for a considerable number of

¹ A summary of this paper was presented at the Tenth Annual Meeting of the American Association for Cancer Research, held in New York on April 5, 1917. Cf. *Jour. Cancer Research*, 1917, ii, 503.

generations the diminution usually following each maximum. This outcome, however, was regarded by them as artificial, indicating neither an increased "virulence" of the tumor cells nor a uniform energy of growth. As a matter of fact, the "virulence" of the cells of any tumor fluctuates, they asserted, between negative and positive phases of growth energy.

Spontaneous tumors, they continued, vary considerably in the ease with which they can be propagated, and these differences had been regarded as degrees of "virulence;" but there exists a certain amount of evidence to show that such neoplasms fluctuate in growth energy in a manner similar to transplantable tumors, and any direct conclusion regarding the "virulence" of a spontaneous tumor drawn from one primary transplantation might accordingly be upset when the same growth gave an opposite result, i.e., when, after having recurred, it had been transplanted a second time.

The term "virulence" thus appeared to these authors an unsuitable one, to be used with so much reservation that it would be better discarded altogether.

Apolant distinguished sharply between two factors comprised in the conception of virulence—*transplantability*, measured by the number of daughter tumors, and *proliferative energy*, evaluated by the rate of growth. Transplantability can be stimulated, according to this investigator, by selecting at each inoculation the most rapidly growing tumor, but the proliferative energy can be hastened, apparently, only to a certain point specific for each strain. To these two qualities Ehrlich added a third, described provisionally as *exhaustive* or *ereptive*; he left open the question, however, whether it rests upon a maximum avidity of the tumor cells for nourishment, or upon some specific relationship toward the materials necessary for the growth of the neoplastic cells.

Apolant did not think that Bashford's hypothesis of adaptation sufficed to explain either the fact that tumors can be made to grow after a time in strange races of mice, or that it is possible to achieve an increase in "virulence." With Ehrlich, he believed that a tumor cell must have a number of potential rudi-

ments besides the actual nutriceptors suited to the food offered by the affected animal. If the growth be transplanted to another host, three occurrences are possible: either the new soil will afford the same nourishment as the old, and the tumor can accordingly grow; or the host will proffer food that none of the nutriceptors is able to grasp, in which case transplantation will be unsuccessful; or, finally, there may be tendered nutriment not exactly like the old, but for which there are present at least the potential rudiments of nutriceptors. In such a case, those cells will die in which the development of potential nutriceptors has not occurred with sufficient promptitude; but the elements which have been stimulated by hunger to produce fresh nutriceptors will be able to survive in the new host, and, furthermore, their proliferation through succeeding generations will be assured, since the newly acquired properties are inheritable.

This hypothesis Apolant regarded as entirely sufficient to explain the sudden changes in growth energy so frequently observed when neoplasms are introduced into strange races, as well as the increase in virulence so often exhibited by tumor cells.

To Haaland (6), also, Ehrlich's explanation appeared eminently suitable. Nevertheless, he regarded the "virulence" as a relative rather than an absolute quantity, since it varies enormously according to the strain of mice inoculated with the tumor in question; thus a new growth so "virulent" as to proliferate in nearly 100 per cent of mice of one strain, may very well prove almost incapable of growth in another.

Loeb (7) has on several occasions expressed the opinion that the increase in growth energy which comes to light after the transplantation of spontaneous tumors, is dependent to a great extent upon the mechanical stimulation of their cells through the process of transplantation.

If our main interest in cancer were reserved for the growth of transplantable tumors, a choice between the terms *virulence* and *adaptation* would be a doctrinaire question indeed. But investigation of the spontaneous tumors of mice will in all probability furnish a number of conclusions that can be transferred to human

neoplasms, which are, after all, the ultimate goal of inquiry, and the question whether or not tumors of the mouse mamma do differ in "virulence" may thus prove to be of really practical importance. But the word *virulence*, with all its tacit suggestion of some analogy between the tumors and the infectious diseases, is an unsuitable one, for which should be substituted some such purely descriptive and noncommittal expression as *proliferative vigor* or *growth energy*.

The evidence already collected appears to suggest that both proliferative energy and adaptation are concerned in the growth of propagable neoplasms. For example, it is well known that mouse tumors exhibit periodical fluctuations in the number of takes from generation to generation, even when they are transplanted into mice of the same strain; and since it is hardly likely that, having once become adapted to a soil, they should lose and regain their adaptability time after time, the most reasonable way of explaining this phenomenon is to refer it to variations in their proliferative energy.

Again, Murray (8) discovered a somewhat similar variability in spontaneous tumors. The percentage of successful grafts obtained by transplanting recurrences of such neoplasms was often entirely different from that following inoculation of the primary tumor; and he found, furthermore, that the behaviour of the spontaneous growth in the animal primarily affected might be almost exactly paralleled by that of the same tumor proliferating in normal animals after transplantation. Rapid recurrence of a primary tumor coincided with an initial rapid proliferation of the transplanted growths, while a long period of quiescence after a second operation was reflected in the transplantation series, where almost every nodule showed a corresponding cessation of growth, and some diminished in size. A second recurrence and renewed rapid growth of the primary tumor was practically synchronous with a similar behavior in the transplanted growths. Russell (9), also, has observed a discrepancy between the inoculation percentage of a tumor and its recurrence, and, like Murray, has ascribed it to cyclic changes in the life of the tumor cell.

All these observations suggest the occurrence of variations in the proliferative energy of the cancer cell, but they do not touch the question of adaptation to a new host. Other evidence, however, seems to show that tumors are able gradually to accustom themselves to nutrient material that is at first more or less foreign, for lying beneath the cyclic variations there is, after all, like a sort of ground-tone, a greater tendency for neoplasms to succeed on removal to another host after they have been under cultivation for a time. That this is true may be safely assumed from Bashford's growth curves (10), of which one (his fig. 6) is here reproduced as an example of the general upward trend accompanying continued propagation (fig. 1). Further evidence of the factor of adaptation is to be found in the observation, substantiated by so many observers, that a spontaneous tumor grows

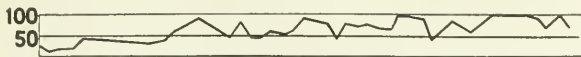


FIG. 1. GRADUAL RISE OF INOCULATION PERCENTAGE

Redrawn from Bashford's figure 6

better when reinoculated into the animal in which it arose, than it does in any other animal; for since there is no reason to suppose that its growth energy varies in the two cases, it must be assumed that it proliferates more successfully in the animal to which it is native because it need make no effort to adapt itself to the soil.

EXPERIMENTS

In order to analyse these two factors, adaptation and growth energy, the following experiments were undertaken; but it is realized, of course, that there may be still other factors involved. The question is: Do some spontaneous mouse tumors succeed upon first transplantation more easily than others because their parenchyma is more plastic as concerns its power to adapt itself to a new host, or merely because they happened to be transplanted at a time when their growth energy was high?

Multiple mammary carcinomata are of frequent occurrence in mice, some of which have as many as seven of these growths.

Suppose now that several spontaneous neoplasms (X, Y, etc.) from the same mouse be inoculated into a series of normal mice from the same source. Since these carcinomata are all composed of tissue from one and the same animal, they should be equally able (or unable) to accustom themselves to an unfamiliar soil; in other words, after having been transplanted, all should produce the same number of daughter tumors, or nearly so, if adaptation were the sole factor controlling the inoculation outcome. If, on the other hand, the number of daughter tumors be conditioned solely by the growth energy of a spontaneous neoplasm at the time of its transplantation, then it is conceivable that primary tumors from the same mouse would vary in their inoculation percentages, and X might give a higher proportion of success than Y, for example.

In order to carry out such an experiment, two assumptions must be made:—that mice from the same dealer offer, in the long run, a soil approximately similar; and that the multiple spontaneous tumors of mice are not metastatic. In regard to the first, it may be asserted that where sufficiently large numbers of animals are employed, individual differences tend to disappear. As for the second, both Gierke (11) and Haaland (12) are of the opinion that a tendency to primary multiplicity exists in the mouse, the latter author advancing, among other reasons, the invariable location of these additional tumors in the mammary apparatus and the wide distances which often separate them, in support of his contention that they are not metastatic.² Murray (13), it is true leaves the question open; but his article was written several years before that of Haaland, and thus at a time when the number of spontaneous tumors that had come under observation at the London institute was not so great as Haaland could command. Hence it would be unfair to advance this as his mature opinion.

² Since this was written, an article by Fischer (*Medicinsk Revue*, 1916) has come to my attention. His experiments, conducted in Haaland's laboratory, show that India ink injected into one mouse mamma never appears in another one. Fischer concludes that cancer cells, therefore, probably do not travel from one mamma to another, and that in all probability multiple tumors in mice are independent (not metastatic) growths.

Nevertheless, in order to reduce to a minimum the chances of error in the following experiments, only those tumors were chosen for transplantation which occupied diagonal positions (e.g., right axilla and left groin) and were thus as remote from one another as they could be, except of course, where more than two neoplasms were used; in such cases this precaution obviously could not be observed.

The tumors from any one mouse were invariably inoculated at one sitting, and by one man, in order to guard against personal variations in technic. This is true in every case except that of mouse $\frac{255}{0}$, which had five tumors; in order that the implantation might be finished within a time roughly comparable to that necessary in the case of other series, five men made the inoculations. It is of interest to note, in passing, that the variations in the case of these five tumors were not so great as occurred in some other series where one man did all the inoculations.

Other precautions were observed to keep the conditions as uniform as possible. Thus, during the implantation of the first tumor the second was kept in the ice-box; 150 mice were engrafted with each tumor in almost every case, in order to eliminate, as far as possible, the fallacies inherent in small numbers; finally, the animals employed for implantation of the growths from any one spontaneously affected mouse were always derived from the same dealer, were of approximately the same age, and were kept after inoculation in the same room and on the same diet. The daughter tumors were charted one month after their implantation and every week thereafter for eight weeks, experience having shown that practically no growths appeared after the lapse of three months. In computing the outcome of an inoculation, it is often difficult to decide whether a mouse with an insignificant stationary or receding tumor is to be included with the positive or the negative animals. Since microscopic examination showed that even the very smallest nodules were composed of healthy tissue, unless undergoing retrogression, it was determined to regard every animal as positive in which any tumor, however small, had appeared, and this even though the graft remained station-

ary or receded; for it was merely the question whether or not the cells of the neoplasm were able to commence growth in another animal that was under investigation, the question of their ultimate success being confused by other factors unconnected with this problem. Nevertheless, the figures thus gained were tested by comparison with others obtained through counting only the progressively growing tumors, when it was found that, although the actual percentages in the former cases were of course higher, the comparative figures remained the same. In other words, of any two or more spontaneous growths from the same mouse, that one which gave the highest percentage of all sorts of daughter tumors (progressively growing, stationary, and receding) also gave the highest percentage of progressively growing ones. Hence the figures represent as close an approach to accuracy as it is possible to obtain in a biological experiment.

Where all the mice in a box were dead at the end of the eighth week (which was not often the case), those remaining alive at the seventh, sixth, fifth, or fourth were counted. But no negative mice dying before the fourth charting were included, though positives which died before this time were.

As the following figures show, 101 spontaneous neoplasms (of which 2 were recurrences) from 44 mice were employed in this investigation. The smallest number from any one animal was 2, and the largest 5. All were carcinomata, except in the case of two mice. In $\frac{310}{0}$, tumor Y was a carcinosarcoma; in $\frac{315}{0}$, tumor Y produced sarcoma in a growth of the first generation, although the slide from the spontaneous neoplasm contained nothing but pure carcinoma. In both cases, however, the differences were much less than often appeared after the transplantation of two carcinomata from the same mouse.

The mere number of daughter tumors may be taken as an index of adaptation and growth energy with comparative safety, without troubling in each individual case to analyze this into the number of receding, stationary, and growing nodules; for it appears to be a general rule that where the percentage of takes is low, most of the tumors will be of insignificant size, and when it is high many of them will grow progressively.

MOUSE	TUMOR	RESULT	SIZE OF TUMORS AND CHARACTER OF THEIR GROWTH	DIFFERENCE
$\frac{112}{0}$	W	$\frac{0}{93} = 0\%$		Less than 10%
	Y	$\frac{0}{85} = 0\%$		
$\frac{159}{0}$	X	$\frac{0}{137} = 0\%$	Pinhead to pea-size. Receding	Less than 10%
	Y	$\frac{2}{106} = 2\%$		
$\frac{145}{0}$	T	$\frac{2}{127} = 2\%$	1 pinhead receding; 1 pea very slowly growing	17%
	U	$\frac{22}{117} = 19\%$	Pinhead to hickory nut. Progressive growth	
$\frac{158}{0}$	X	$\frac{0}{82} = 0\%$	Pea-size. Receding	Less than 10%
	Y	$\frac{1}{69} = 1\%$		
	X (recurrence)	$\frac{3}{60} = 5\%$	Small pea. Stationary or receding	Less than 10%
	Y (recurrence)	$\frac{5}{49} = 10\%$	Small pea. Stationary or receding	
$\frac{182}{0}$	W	$\frac{0}{66} = 0\%$	Pinhead to hickory nut. Progressive	30%
	X	$\frac{43}{141} = 30\%$		
	Y	$\frac{4}{79} = 5\%$	Pinhead to pea. Receding	
$\frac{202}{0}$	X	$\frac{1}{82} = 1\%$	Pea-size. Progressive	Less than 10%
	Y	$\frac{8}{106} = 7\%$	Hazelnut to walnut. Progressive	

MOUSE	TUMOR	RESULT	SIZE OF TUMORS AND CHARACTER OF THEIR GROWTH	DIFFERENCE
$\frac{206}{0}$	X	$\frac{4}{107} = 4\%$	Pinhead. Receding	Less than 10%
	Y	$\frac{7}{79} = 9\%$	Pinhead to pea. Receding	
$\frac{212}{0}$	X	$\frac{23}{91} = 25\%$	Pinhead to cherry-stone. Receding	Less than 10%
	Y	$\frac{29}{117} = 25\%$	Pinhead to hazelnut. Majority progressive	
$\frac{200}{0}$	W	$\frac{3}{108} = 3\%$	Pinhead to pea. Receding	17%
	X	$\frac{22}{109} = 20\%$	Pinhead to pea. Majority receding	
$\frac{234}{0}$	X	$\frac{0}{93} = 0\%$		Less than 10%
	Y	$\frac{6}{84} = 7\%$	Pinhead to pea. Receding	
$\frac{251}{0}$	X	$\frac{22}{94} = 23\%$	Pinhead to hazelnut. Majority stationary	11%
	Y	$\frac{8}{67} = 12\%$	Pinhead to pea. Receding	
$\frac{255}{0}$	T	$\frac{0}{38} = 0\%$		28%
	U	$\frac{7}{64} = 11\%$	Pinhead to small pea. Receding	
	W	$\frac{12}{43} = 28\%$	Pinhead to cherry-stone. Majority stationary	
	X	$\frac{0}{79} = 0\%$		
	Y	$\frac{5}{74} = 7\%$	Pinhead to pea. Receding	

MOUSE	TUMOR	RESULT	SIZE OF TUMORS AND CHARACTER OF THEIR GROWTH	DIFFERENCE
$\frac{275}{0}$	X	$\frac{0}{63} = 0\%$	Pinhead receding to hazelnut slowly progressive	25%
	Y	$\frac{16}{65} = 25\%$		
$\frac{315}{0}$	X	$\frac{0}{72} = 0\%$	Pea receding to walnut progressive	20%
	Y	$\frac{14}{68} = 20\%$		
$\frac{370}{0}$	X	$\frac{10}{86} = 12\%$	Pinhead. Stationary or receding	10%
	Y	$\frac{2}{81} = 2\%$	Pinhead. Receding	
$\frac{349}{0}$	X	$\frac{21}{77} = 27\%$	Majority pinhead to pea receding. Two progressive	15%
	Y	$\frac{9}{77} = 12\%$	Pinhead. Receding	
$\frac{375}{0}$	X	$\frac{4}{69} = 6\%$	Pinhead. Receding	Less than 10%
	Y	$\frac{0}{55} = 0\%$		
$\frac{396}{0}$	X	$\frac{3}{63} = 5\%$	Pinhead. Receding	Less than 10%
	Y	$\frac{0}{43} = 0\%$		
$\frac{394}{0}$	W	$\frac{18}{85} = 21\%$	Pinhead to pea. Receding	Less than 10%
	X	$\frac{17}{110} = 15\%$	Majority pinhead to pea receding	
	Y	$\frac{15}{95} = 16\%$	Pinhead to pea. Receding	

MOUSE	TUMOR	RESULT	SIZE OF TUMORS AND CHARACTER OF THEIR GROWTH	DIFFERENCE
$\frac{445}{0}$	X	$\frac{26}{57} = 46\%$	Pea to hickory nut. Progressive	11%
	Y	$\frac{37}{65} = 57\%$	Pea to walnut. Progressive	
$\frac{474}{0}$	X	$\frac{2}{74} = 3\%$	1 pea receding; 1 hazelnut progressive	49%
	Y	$\frac{48}{93} = 52\%$	Pea to hickory nut. Progressive	
$\frac{559}{0}$	X	$\frac{43}{88} = 49\%$	Pea to hickory nut. Progressive	Less than 10%
	Y	$\frac{45}{97} = 46\%$	Pea to hickory nut. Progressive	
$\frac{463}{0}$	X	$\frac{11}{73} = 15\%$	4 hazel to hickory nut	12%
	Y	$\frac{2}{59} = 3\%$	Pinhead	
$\frac{645}{0}$	X	$\frac{1}{46} = 2\%$	Pinhead	Less than 10%
	Y	$\frac{1}{33} = 3\%$	Pinhead	
$\frac{605}{0}$	X	$\frac{0}{47} = 0\%$		41%
	Y	$\frac{24}{58} = 41\%$	Majority progressively growing, walnut size	
$\frac{809}{0}$	X	$\frac{34}{97} = 35\%$	Pinhead to hickory nut. Receding	34%
	Y	$\frac{1}{96} = 1\%$	Pinhead. Receding	
$\frac{786}{0}$	X	$\frac{2}{48} = 4\%$	Pinhead	Less than 10%
	Y	$\frac{4}{91} = 4\%$	Pinhead	

MOUSE	TUMOR	RESULT	SIZE OF TUMORS AND CHARACTER OF THEIR GROWTH	DIFFERENCE
$\frac{827}{0}$	V	$\frac{100}{128} = 78\%$	Majority progressively growing, hickory nut size	62%
	W	$\frac{60}{109} = 55\%$	A few reached hazelnut size. Majority receded	
	X	$\frac{15}{94} = 16\%$	Pinhead	
$\frac{770}{0}$	X	$\frac{7}{86} = 8\%$	Hazelnut to hickory nut size, progressing or receding	Less than 10%
	Y	$\frac{9}{112} = 8\%$	Pea to hickory nut, progressing or receding	
$\frac{828}{0}$	X	$\frac{18}{100} = 18\%$	Majority pinhead. Remainder pea size	Less than 10%
	Y	$\frac{9}{102} = 9\%$	Pinhead	
$\frac{841}{0}$	T	$\frac{19}{83} = 23\%$	Pinhead	23%
	W	$\frac{0}{76} = 0\%$		
	X	$\frac{26}{112} = 23\%$	Majority pinhead	
$\frac{875}{0}$	W	$\frac{14}{90} = 15\%$	Majority pinhead	Less than 10%
	X	$\frac{17}{71} = 24\%$	Majority pinhead	
$\frac{847}{0}$	X	$\frac{9}{139} = 6\%$	Pinhead	Less than 10%
	Y	$\frac{0}{123} = 0\%$		
$\frac{788}{0}$	X	$\frac{0}{94} = 0\%$		Less than 10%
	Y	$\frac{1}{77} = 1\%$	Pinhead	

MOUSE	TUMOR	RESULT	SIZE OF TUMORS AND CHARACTER OF THEIR GROWTH	DIFFERENCE
$\frac{856}{0}$	V	$\frac{0}{115} = 0\%$	Pinhead	Less than 10%
	X	$\frac{4}{92} = 4\%$		
	Y	$\frac{0}{110} = 0\%$		
$\frac{767}{0}$	T	$\frac{63}{128} = 49\%$	Pinhead to pea. Many receding	18%
	V	$\frac{35}{113} = 31\%$	Pinhead to pea. Many receding	
	Y	$\frac{75}{147} = 51\%$	Majority pea to hickory nut, and progressively growing	
$\frac{902}{0}$	W	$\frac{0}{70} = 0\%$		Less than 10%
	X	$\frac{0}{66} = 0\%$		
$\frac{803}{0}$	W	$\frac{1}{88} = 1\%$	Pinhead	10%
	Y	$\frac{11}{96} = 11\%$	Pinhead to pea. Majority receding	
$\frac{838}{0}$	V	$\frac{1}{107} = \frac{1}{10}\%$	Pinhead	Less than 10%
	X	$\frac{2}{97} = 2\%$	Pinhead	
$\frac{944}{0}$	X	$\frac{16}{123} = 13\%$	Pinhead to pea, all receding but 1	18%
	Y	$\frac{39}{126} = 31\%$	Pinhead, all receding but 3	

MOUSE	TUMOR	RESULT	SIZE OF TUMORS AND CHARACTER OF THEIR GROWTH	DIFFERENCE
$\frac{1069}{0}$	V	$\frac{9}{123} = 7\%$	Pinhead to small pea. Receding	Less than 10%
	W	$\frac{0}{113} = 0\%$		
	X	$\frac{5}{114} = \frac{9}{10}\%$	Pea to hickory nut. Progressive	
$\frac{925}{0}$	W	$\frac{0}{97} = 0\%$		Less than 10%
	X	$\frac{0}{96} = 0\%$		
	Y	$\frac{0}{83} = 0\%$		
$\frac{1241}{0}$	X	$\frac{1}{96} = 1\%$	Pea size	10%
	Y	$\frac{10}{93} = 11\%$	Pea to hickory nut	
$\frac{1269}{0}$	W	$\frac{0}{70} = 0\%$		Less than 10%
	X	$\frac{7}{103} = 7\%$	Pea to walnut. Progressive	

It is evident from the table that spontaneous growths from the same mouse give in many cases about the same percentage of daughter tumors after transplantation, though in some instances there is considerable difference in the result. It is impossible to say, however, just where the line shall be drawn between a similar and a dissimilar outcome, for it is difficult to decide what margin to allow for unavoidable experimental error. To those familiar with the enormous differences produced in biological experiments by factors that are not under the control of the investigator, 10 per cent will seem a low point at which to place the dividing line. Yet even when spontaneous tumors are

regarded as having given a similar inoculation percentage if the difference between the highest and the lowest outcome be not greater than 10 per cent, it appears that the growths of more than half (27) of the 44 mice behaved similarly after transplantation. If the more reasonable difference of 25 per cent be chosen as the point at which to divide similarly from dissimilarly growing tumors, it is found that the spontaneous neoplasms of 38 out of 44 mice resembled one another in their power (or lack of power) to grow after transplantation; in other words, multiple tumors from 38 mice out of 44 exhibited a difference of but 25 per cent or less in the number of daughter tumors. Finally, if only those tumors be regarded as similar which show a difference between their inoculation results of 50 per cent or less, the growths of 42 mice out of 44 fall in this class—for $\frac{474}{0}$ approaches so closely to 50 per cent that it may be eliminated with $\frac{827}{0}$.

Thus, when all inoculation results below 25 per cent are regarded as equal, spontaneous neoplasms from approximately nine-tenths of the mice are found to yield the same outcome after transplantation; this suggests that adaptation is an important factor in the establishment of daughter tumors, and the hypothesis is strengthened by the fact that the growths from more than half the mice still show a similar number of daughter tumors when only those percentages are declared equal which vary by 10 per cent or less.

Two mice were found, however, whose tumors gave inoculation results differing by as much as 49 and 62 per cent respectively, which indicates that spontaneous neoplasms may exhibit variations in their growth energy after transplantation. Such a supposition is borne out by mouse $\frac{827}{0}$, where three grades of proliferative energy appear to have been present in her three growths, shown not only by the inoculation percentage but also by the manner in which the daughter tumors grew. Thus X produced 16 per cent of receding daughter tumors, none larger than a pinhead; W gave rise to 55 per cent of pea-size growths, of which a few reached the dimensions of a hazelnut though the majority receded; and V afforded 78 per cent of tumors, of which the majority grew progressively and had reached the size of a hickory nut at the end of eight weeks.

Such variations, however, do not appear to be the rule, for in only 6 mice out of 44 did the inoculation percentages of multiple neoplasms vary by more than 25 per cent.

Hence it is probable that the factor which usually decides whether or not a spontaneous tumor can be successfully transplanted, is adaptation. But it is equally probable that multiple spontaneous neoplasms occasionally vary in their proliferative energy; in most of them this is so low after transplantation as to be masked by a failure on the part of the tumor cells to adapt themselves, though in the case of a few growths it rises so high as to transcend the adaptive power of these elements.

It may be pointed out, in passing, that the recurrences in mouse $\frac{158}{9}$ furnished approximately the same proportion of daughter tumors as the primary growths, and that no parallel could be observed between the proliferative rate of spontaneous neoplasms in the mouse affected, and the percentage of daughter tumors. The more slowly growing tumor sometimes produced the greater percentage.

SUMMARY

The presence (or absence) of power to adapt themselves to new hosts appears to be a deciding factor in the success (or failure) of most spontaneous mouse carcinomata after transplantation. In a few such neoplasms, however, a high proliferative energy appears to aid in gaining a foothold.

REFERENCES

- (1) EHRlich, P.: Arb. a. d. königl. Inst. für exp. Therap. zu Frankfurt a. M., 1906, Heft 1, 90.
Ztschr. f. Krebsforsch., 1907, v, 62.
- (2) APOLANT, H.: Ztschr. f. allg. Physiol., 1909, ix, Sammelreferat, 69.
- (3) BASHFORD, E. F., MURRAY, J. A., HAALAND, M., and BOWEN, W. H.: Third Sci. Report, Imperial Cancer Research Fund, London, 1908, pp. 109, 161, 270.
- (4) MURRAY, J. A.: Third Sci. Report, Imperial Cancer Research Fund, London, 1908, pp. 109, 161.
- (5) BASHFORD, E. F., MURRAY, J. A., and CRAMER, W.: Proc. Roy. Soc., Series B, 1907, lxxix, 164.
Third Sci. Report, Imperial Cancer Research Fund, London, 1908, pp. 326, 337.

- (6) HAALAND, M.: Berl. klin. Wehnschr., 1907, xliv, 713.
- (7) FLEISHER, M. S., and LOEB, L.: Jour. Med. Research, 1916, n. s. xxix, 1.
- (8) MURRAY, J. A.: Third Sci. Report, Imperial Cancer Research Fund, London, 1908, p. 106.
- (9) RUSSELL, B. R. G.: Jour. Path. and Bacteriol., 1910, xiv, 347.
- (10) BASHFORD, E. F.: Fourth Sci. Report, Imperial Cancer Research Fund, London, 1911, p. 131.
- (11) GIERKE, E.: Third Sci. Report, Imperial Cancer Research Fund, London, 1908, p. 118.
- (12) HAALAND, M.: Fourth Sci. Report, Imperial Cancer Research Fund, London, 1911, p. 25.
- (13) MURRAY, J. A.: Third Sci. Report, Imperial Cancer Research Fund, London, 1908, p. 98.

A MAMMARY CARCINOMA IN THE CAT

SHIGEMITSU ITAMI

*From Columbia University, George Crocker Special Research Fund, F. C. Wool,
Director*

Received for publication, January 2, 1918

Mammary carcinomata of the cat are not of particularly rare occurrence, for a number of cases are to be found in the literature of the past twenty-seven years. One of these was reported by Spence (1), five by Sticker (2), one by Petit (3), one by Leyden (4), one by Petit and Cornil (5), four by Murray (6), one by Petit and Finzi (7), and one by Clumet (8). To these may be added a few cases cited by Wolff (9), and by Petit (10), whose original articles, however, are not available to the writer.

The example to be described in this paper is that of a large female cat received through the kindness of Dr. Benjamin White of Otisville, N. Y., on March 23, 1917.

In the right axillary mamma there was a hard, nodular, tumor about 10 by 3 by 2 cm., containing a few small cysts, and adherent to the skin which, toward the lower pole of the growth, presented two ulcers involving the lowermost axillary nipple. These resembled cancerous ulcers in the human subject, their edges being raised and scalloped and the floor formed of firm granulation tissue discharging a foul ichor. Toward the upper pole, the tumor grew narrow, passing by the upper nipple and extending into the right axilla where it broke up into a string of separate nodules each about the size of a green pea.

At autopsy, on March 26, 1917, it was found that the growth was not adherent to the underlying musculature. The axillary lymph-nodes, as well as the supracondylar and one in the neighboring connective tissue, were enlarged. The lungs contained a few shot-sized nodules, these being more prevalent in the middle lobe of the right lung, which appeared to be carnified and con-

tained about six such lesions. Except for one or two small shot-like nodules in the liver, which might have been metastases, the rest of the body was free from secondary growths. There was one fetus in the right horn of the uterus.

Sections removed from the tumor appear upon microscopic examination to be composed of papillary cysts with a stroma of thickened connective tissue. The cysts are almost completely filled by a considerable number of elongated processes (fig. 1)



FIG. 1. SHOWING PAPILLARY NATURE OF THE TUMOR

springing from their walls, which branch out irregularly and appear to anastomose with one another. The walls of the cysts are made up of well-defined connective tissue lined with epithelium, the elements of which consist of large cells ranging in shape from cuboidal to cylindrical, usually arranged in one layer, and containing large oval nuclei; in some places, however, especially at the bases of the papillae, the epithelial lining is exceedingly thick. Mitotic figures are prominent.

While papillary outgrowth is so luxuriant, invasion of the surrounding tissue (fig. 2) is also a noteworthy feature, the subjacent lymph-channels, or perhaps the connective tissues underlying the bases of the papillae, being frequently attacked.

The stroma of the tumor is abundant and firm, forming a prominent part of the growth; in some portions it is so dense as to suggest scar tissue. The fibers are arranged in irregular bands, and it is from those forming the walls of the cysts that fibrils to the papillae branch out. There are many areas of circumscribed round cell infiltration throughout the stroma. The tumor has no capsule and fades out indefinitely into the surrounding tissues.

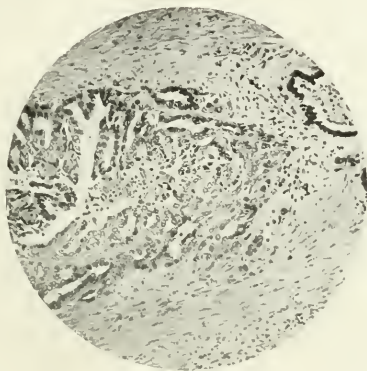


FIG. 2. INVASION OF THE SURROUNDING TISSUES

In the supracondylar nodule above referred to, the lymphoid tissue of the lymph-node is displaced by tumor.

The pulmonary metastases contained few cysts with intracystic projections and well-defined walls, but the dominant feature here is that of a papillary carcinoma without cyst formation. The papillae grow into and fill the air spaces of the lung, without actual involvement of their wall, and except for a moderate dilation of the blood-vessels and a slight round-cell infiltration, the interlobular tissue around the nodules remains unaffected.

The nodules in the liver prove to be fatty infiltration.

The corresponding mamma on the opposite side, which did not show any macroscopic changes at autopsy, was examined microscopically to see whether changes like those described by Haaland (11) in the mouse could be discovered. The coexisting pregnancy naturally obscured somewhat the conditions present in the organ, but there could be no doubt that it was the seat of an extensive papillary cystadenomatous change, or that certain regions were carcinomatous; whether these areas represented new tumors, however, or metastases from that in the other breast, could not be decided.

REFERENCES

- (1) SPENCER, W. G.: *Tr. Path. Soc.*, London, 1890, xli, 400.
- (2) STICKER, A.: *Ueber den Krebs der Tiere* (Sonder Abdruck aus dem *Arch. f. klin. Chir.*, Bd. 65), Berlin, 1902, pp. 79 and 126.
- (3) PETIT, G.: *Bull. Soc. anat. de Paris*, 1902, iv, 409.
- (4) LEYDEN, E. VON: *Ztschr. f. klin. Med.*, 1904, lli, 409.
- (5) PETIT, G. AND CORNIL, V.: *Bull. Soc. anat. de Paris*, 1906, lxxxi, 208.
- (6) MURRAY, J. A.: *Third Sci. Report, Imperial Cancer Research Fund*, London, 1908, p. 44.
- (7) PETIT, G. AND FINZI, G.: *Bull. de l'Assoc. franç. pour l'étude du cancer*, Paris, 1910, vii, 305.
- (8) CLUNET, J.: *Recherches expérimentales sur les Tumeurs malignes*. Paris, 1910, p. 4.
- (9). WOLFF, J.: *Die Lehre von der Krebskrankheit*, Jena, 1913, Teil iii, 1ste Abt., 253.
- (10) PETIT, G.: *Bull. Soc. centr. de Méd. vét.*, 1914-15.
- (11) HAALAND, M.: *Fourth Sci. Report, Imperial Cancer Research Fund*, London, 1911, 30-37.

AN INVESTIGATION OF THE POWER OF MESODERMAL DERIVATIVES TO IMMUNIZE MICE AGAINST TRANSPLANTABLE TUMORS

SHIGEMITSU ITAMI

*From Columbia University, George Crocker Special Research Fund, F. C. Wood,
Director*

Received for publication, January 21, 1918

When the various tissues which have been used to immunize mice against transplantable tumors are passed in review, it is found that representatives of all three germ layers have the power to confer resistance, with the exception of one ectodermal derivative, i.e., the crystalline lens. While both Schöne (1) and Borrel and Bridré (2) have asserted that the injection of testis will not bring about the refractory state, a more recent investigator (3) believes that the testis will confer resistance; furthermore, an experiment conducted in this laboratory by Drs. Bullock and Rohdenburg, and hitherto unpublished, shows that the testis is not without immunizing power, at least in the rat. It has, therefore, been entered in the table (table 3) accordingly (+).

The failure of mouse lens to produce immunity in the experiment of Uhlenhuth and Weidanz (4) is an interesting phenomenon in view of the constancy with which most other tissues call forth the refractory state. Its inactivity cannot be ascribed to an insufficient amount, at any rate, for three doses (1 cc. each) of an emulsion were given; nor was tumor inoculation undertaken at an unfavorable time, for it followed twenty-eight days after the first treatment with lens, and eight days after the last, a period within which immunity should still be high, as Woglom (5) has shown.

The experiment was accordingly repeated by the present writer in another species of animal, rats treated with 0.05 cc.

of an emulsion of rat lens, being inoculated one, two, three, or four weeks afterwards with 0.003 gram¹ of the Flexner-Jobling rat carcinoma by the needle method. The outcome was identical with that recorded by Uhlenhuth and Weidanz, for not the slightest indication of immunity could be discovered in any of the experiments, of which figure 1 reproduces a perfect sample.

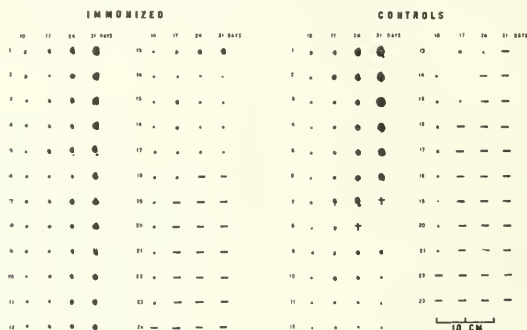


FIG. 1. EXPERIMENT $\frac{\text{FRC}}{19\text{C}}$

No immunity to carcinoma three weeks after treatment with 0.05 cc. of an emulsion of rat lens. Tumor dose, 0.003 gram by the needle method.

It may be objected that the amount of lens administered was too small. But the experience of Bullock and Rohdenburg (6) shows that this quantity of rat embryo skin emulsion will produce a distinct resistance against the same dose (0.003 gram) of the same tumor (Flexner-Jobling rat carcinoma). Still it is possible that the amount of lens, while not actually too small, was relatively insufficient; for the lens contains but a few cells, and the authors (6) just cited have demonstrated that tissues like cartilage and bone, which have a minimum of cellular material

¹ In previous publications from the Imperial Cancer Research Fund and from this laboratory, the inoculation dose, when the needle method was used, has been estimated as 0.01 or 0.02 gram; but such grafts have recently been found, as a matter of fact, to weigh about 0.002 and 0.003 gram respectively.

and a maximum of matrix, do not produce a high resistance. It may be, therefore, that the lens fails for a similar reason, and the comparative failure of all three may perhaps prove to be another vindication of Haaland's assertion (7) that growth of the cells after their introduction is an essential for the production of immunity.

It will be appreciated that the preparation of air emulsion of rat lens in sufficient amount is an expensive and laborious process, and that for this reason a large quantity could not be obtained at the present time owing to war conditions; it may be possible at some future time, however, to repeat one of these experiments, using a larger dose.

The brain will perhaps have to be included among the tissues incapable of eliciting distinct resistance; for, while Borrel and Bridré (8) are said to have succeeded in immunizing with brain, a repetition of the experiment at this laboratory has given entirely negative results. Since the French observers conducted their investigation some time ago, when the importance of dosage and time interval was hardly yet recognized, these more recent experiments, in which both have been taken into account, afford a result that is, perhaps, nearer the truth. Accordingly, the brain has been entered in table 3 as possibly negative, a definite decision, as in the case of the lens, being reserved for the future.

The following experiments were undertaken to determine whether any other tissues share with the lens, brain, cartilage, and bone, their inability to elicit a vigorous immunity. Two mesodermal derivatives—muscle and lymph-node—were chosen. In order that the findings might not be vitiated by the presence of blood in these tissues, the greatest care was taken not to injure large vessels during the removal of the material; the small amount of blood contained in the muscle and the lymph-node themselves may be regarded as insufficient to produce any immunity.

Muscles or lymph-nodes were removed from healthy mice, emulsified, and administered subcutaneously in the left axilla of normal young adult mice in doses of 0.05 cc. (lymph-node) and 0.1 cc. (muscle). Into the opposite axillae of the animals

thus injected, and into untreated normal controls of the same size and breed which had been kept aside under identical conditions, grafts (0.003 gram) of tumor were implanted one to four weeks after the preliminary treatment. The three tumors employed, 63, 11 and 48, are transplantable mammary adenocarcinomata.

Daughter tumors were charted first ten days after the inoculation of the grafts, and subsequently at intervals of one week, and the results thus obtained are reproduced in a number of charts and a table (table 1). These, however, represent but a few of the experiments; it is unnecessary to publish the whole series because the results were similar in all cases.

It will be seen that preliminary treatment with lymph-node calls forth a distinct resistance (figs. 2 to 5) to two carcinomata, though the immunity is perhaps of shorter duration than that induced by some other tissues. The vigorous refractory state obtained with lymph-node, however, obviously has no bearing upon questions regarding the rôle of the lymphocyte in immunity, for an equally high protection can be brought about with other materials.

Muscle, on the other hand, does not induce resistance in the amount employed (figs. 6 to 8). This material, therefore, appears to share with bone, cartilage and the crystalline lens their inability to evoke an efficient immunity against cancer. Why this should be so is not clear; certainly the proportion of cells in muscle is greater than in bone, cartilage, or lens. It may be that the cells of muscle are not readily broken down and absorbed after introduction into the subcutaneous tissues.

The fact having been established that muscle fails to elicit immunity to carcinoma, it becomes necessary to know whether it will confer resistance against a growth which is, like itself, of mesodermal origin, or whether it will resemble such an efficient immunizer against carcinoma as epithelium is known to be, in being unable to produce resistance against the majority of sarcomata. Mice that had been treated with muscle were therefore tested with two sarcomata, the Ehrlich and the Crocker Fund No. 180, both known to be insusceptible to 0.1 cc. of embryo

TABLE I

CARCIN- OMA	INTERVAL	MICE	TREATED WITH					
			Muscle			Lymph-node		
			Survived	Negative		Survived	Negative	
				Number	Per cent		Number	Per cent
63	1 week	Treated	22	5	22.72	23	21	91.30
		Controls	22	1	4.58	22	1	4.58
	2 weeks	Treated	22	7	31.81	22	18	81.81
		Controls	21	1	4.76	21	4	19.04
	3 weeks	Treated	18	5	27.77	23	7	30.43
		Controls	24	2	8.33	23	0	0.00
	4 weeks	Treated	24	9	37.50	16	3	18.75
		Controls	24	4	16.66	24	2	8.33
11	1 week	Treated	23	6	26.08	10	10	100.00
		Controls	24	8	33.33	10	5	50.00
	2 weeks	Treated	18	6	33.33	20	20	100.00
		Controls	24	6	25.00	22	9	40.00
	3 weeks	Treated	20	7	35.00	9	8	88.88
		Controls	17	8	47.05	12	4	33.33
	4 weeks	Treated	23	7	30.43	22	18	81.81
		Controls	23	10	43.47	24	8	33.33
48	1 week	Treated	23	11	47.82	23	19	82.60
		Controls	22	6	27.27	23	1	4.34
	2 weeks	Treated	21	18	38.09	22	18	81.81
		Controls	23	0	0.00	10	4	20.00
	3 weeks	Treated	19	6	31.59	18	15	83.33
		Controls	21	7	33.33	17	7	41.17
	4 weeks	Treated				19	13	68.42
		Controls				21	3	14.28

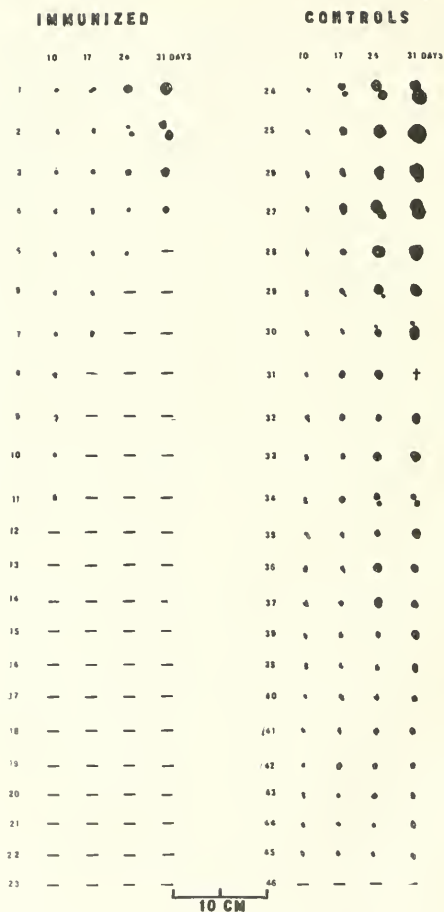


FIG. 2. EXPERIMENT $\frac{48}{33B}$

Distinct immunity to carcinoma one week after treatment with 0.05 cc. of an emulsion of mouse lymph-node. Tumor dose, 0.003 gram by the needle method.



FIG. 3. EXPERIMENT $\frac{63}{136L}$

Distinct immunity to carcinoma one week after treatment with 0.05 cc. of an emulsion of mouse lymph-node. Tumor dose, 0.003 gram by the needle method.

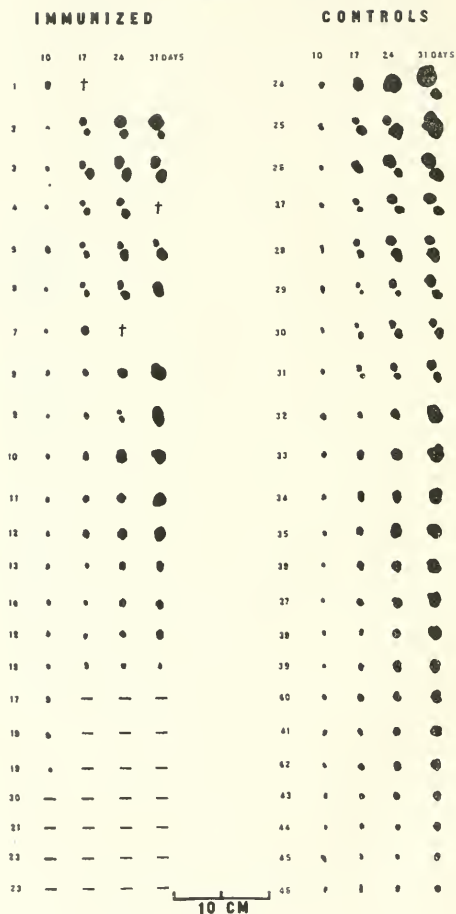


FIG. 4. EXPERIMENT $\frac{63}{141H}$

Slight immunity to carcinoma three weeks after treatment with 0.05 cc. of an emulsion of mouse lymph-node. Tumor dose, 0.003 gram by the needle method.

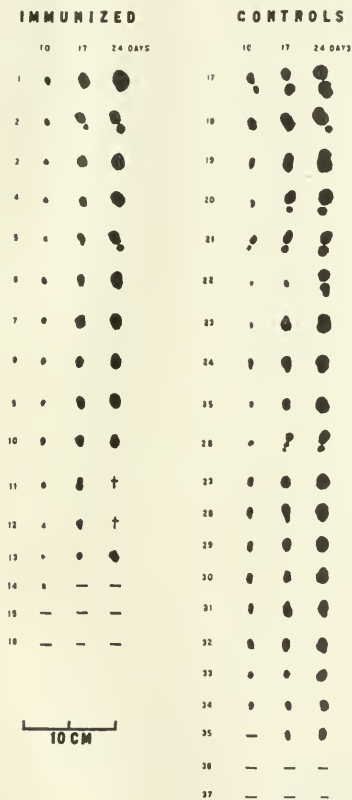


FIG. 5. EXPERIMENT $\frac{63}{13SQ}$

Practically no immunity to carcinoma four weeks after treatment with 0.05 cc. of an emulsion of mouse lymph-node. Tumor dose, 0.003 gram by the needle method.

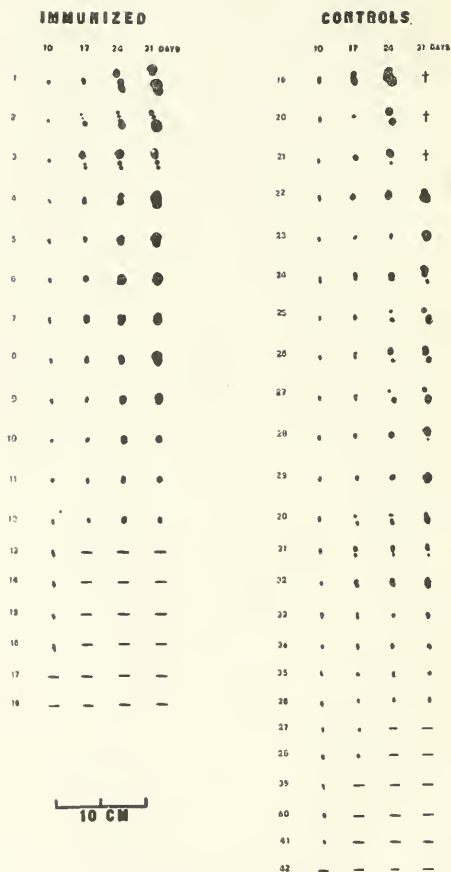
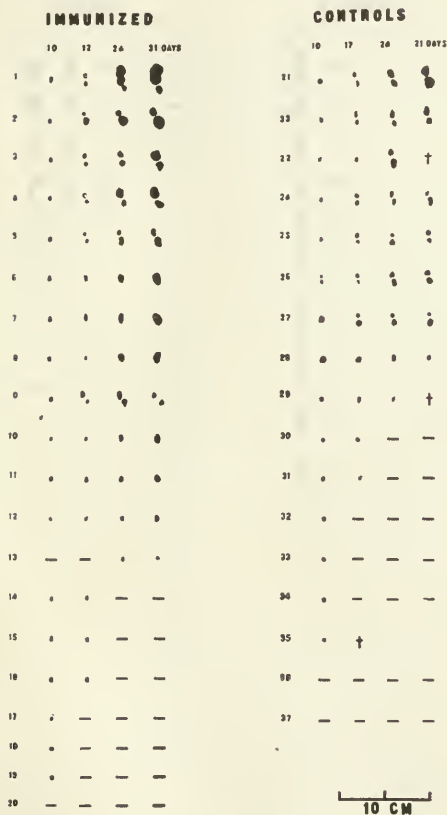
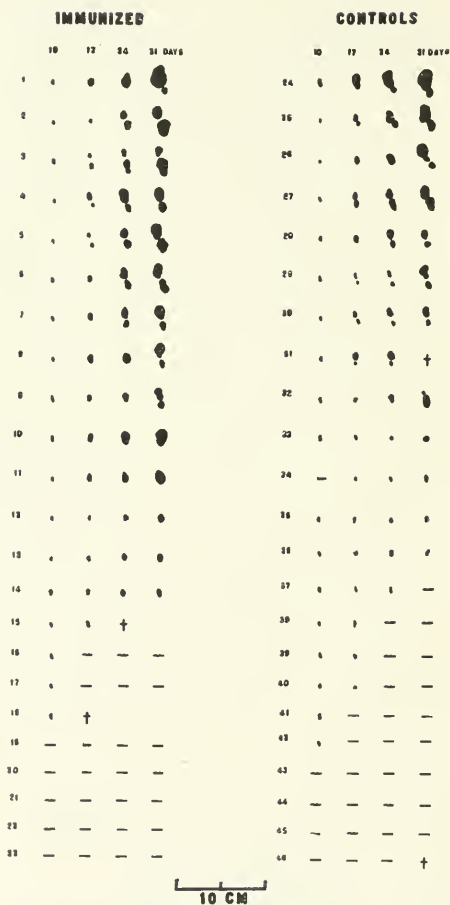


FIG. 6. EXPERIMENT $\frac{11}{44E}$

No immunity to carcinoma two weeks after treatment with 0.1 cc. of an emulsion of mouse muscle. Tumor dose, 0.003 gram by the needle method.

FIG. 7. EXPERIMENT $\frac{11}{44B}$

No immunity to carcinoma three weeks after treatment with 0.1 cc. of an emulsion of mouse muscle. Tumor dose, 0.003 gram by the needle method.

FIG. 8. EXPERIMENT $\frac{11}{44D}$

No immunity to carcinoma four weeks after treatment with 0.1 cc. of an emulsion of mouse muscle. Tumor dose, 0.003 gram by the needle method.

skin (figs. 10 and 12, and "Treated controls" in table 2). As figures 9 to 13 and table 2 show, muscle is impotent to protect against sarcoma, and the same is true of lymph-node. In other words, the mesodermal derivatives chosen do not protect against two neoplasms of mesodermal origin, although the interval

TABLE 2

SARCOMA	INTERVAL	MICE	TREATED WITH					
			Muscle			Lymph-node		
			Survived	Negative		Survived	Negative	
				Num-ber	Per cent		Num-ber	Per cent
180	1 week	Treated	21	6	28.75	24	11	45.83
		Controls	23	1	4.34	21	2	9.52
	2 weeks	Treated	22	4	18.18	20	0	0.00
		Controls	24	0	0.00	21	0	0.00
		"Treated controls"				15	0	0.00
	3 weeks	Treated	14	1	7.14	17	2	11.76
		Controls	20	0	0.00	20	2	10.00
		"Treated controls"	22	8	36.36			
	4 weeks	Treated	33	2	6.06	11	0	0.00
		Controls	35	1	2.85	20	1	5.00
		"Treated controls"				14	1	7.14
	E. S.	1 week	Treated	24	0	0.00	16	1
Controls			19	0	0.00	19	0	0.00
"Treated controls"			19	0	0.00			
2 weeks		Treated	23	0	0.00	22	0	0.00
		Controls	22	1	4.58	22	1	4.58
		"Treated controls"	22	4	18.18			

between treatment and tumor inoculation was inside the limits of the period during which immunity to sarcoma can sometimes be elicited (9).

In table 3 all the tissues that have now been tested as to their immunizing power are arranged according to derivation. The failure of cartilage, bone, and muscle is not due to their meso-

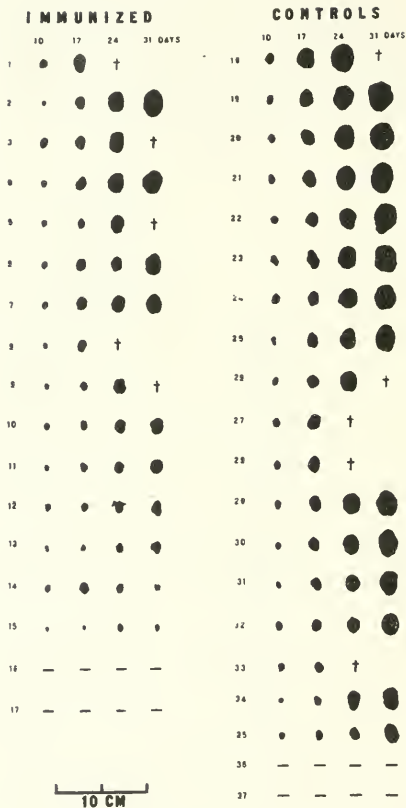


FIG. 9. EXPERIMENT $\frac{180}{26H}$

No immunity to sarcoma three weeks after treatment with 0.05 cc. of an emulsion of mouse lymph-node. Tumor dose, 0.003 gram by the needle method.

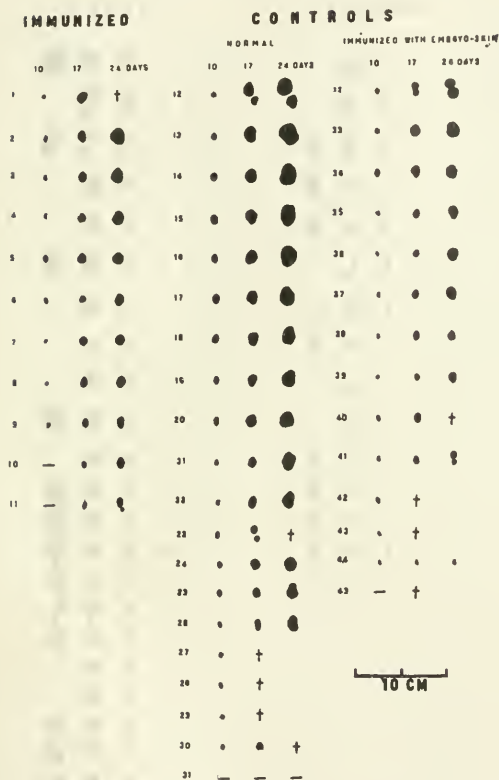


FIG. 10. EXPERIMENT $\frac{180}{431}$

No immunity to sarcoma four weeks after treatment with 0.05 cc. of an emulsion of mouse lymph-node. Tumor dose, 0.003 gram by the needle method. It will be noted that embryo skin also fails to produce resistance against this sarcoma.

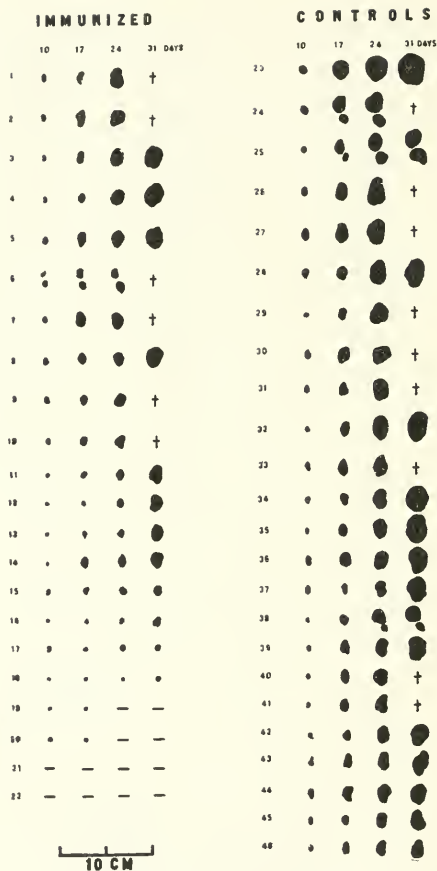
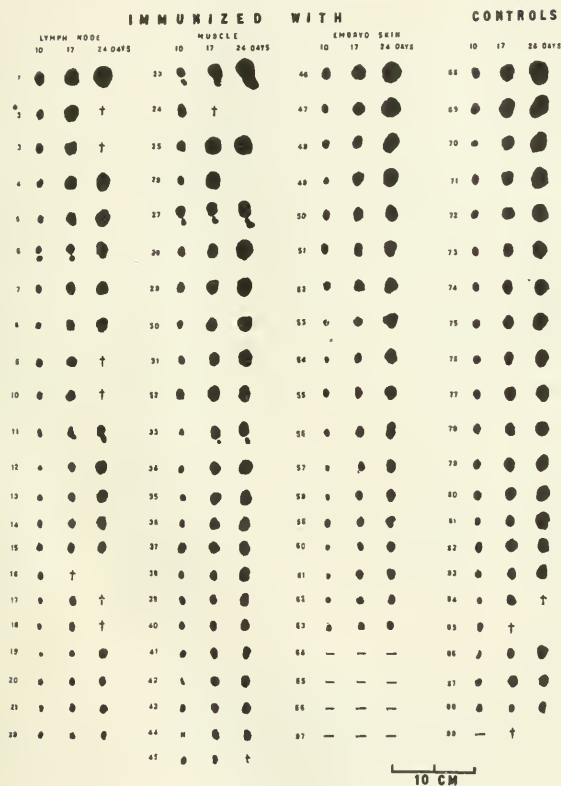


FIG. 11. EXPERIMENT $\frac{180}{26D}$

No immunity to sarcoma two weeks after treatment with 0.1 cc. of an emulsion of mouse muscle. Tumor dose, 0.003 gram by the needle method.

FIG. 12. EXPERIMENT $\frac{ES}{60G}$

No immunity to sarcoma two weeks after treatment with 0.1 cc. of an emulsion of mouse muscle. Tumor dose, 0.003 gram by the needle method. It will be noted that lymph-node and embryo skin also fail to produce resistance against this sarcoma.

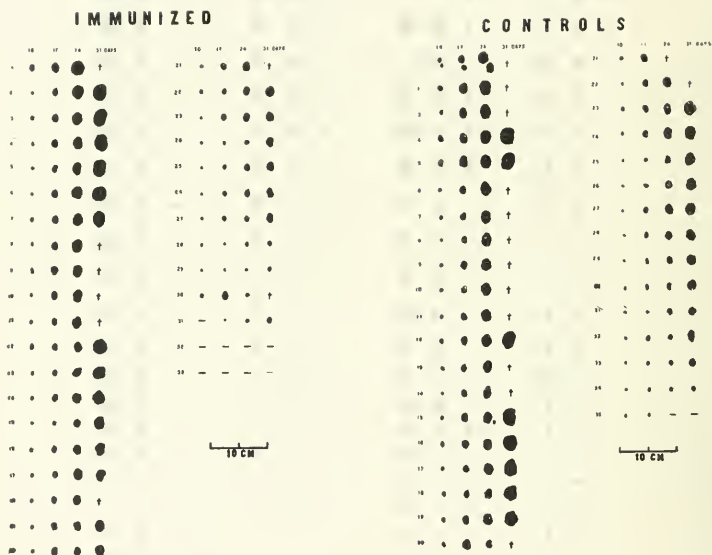


FIG. 13. EXPERIMENT $\frac{180}{26F}$

No immunity to sarcoma four weeks after treatment with 0.1 cc. of an emulsion of mouse muscle. Tumor dose, 0.003 gram by the needle method.

dermal origin, for other materials derived from the same source are fully potent; furthermore, the inability is shared by at least one tissue descended from the ectoderm. As has already been pointed out, lens, cartilage, and bone contain comparatively few cells in contrast with those tissues which are efficient in eliciting the refractory state; yet muscle, which is more cellular, also fails completely. This can hardly be referred to its high differentiation, for the skin, which is the most active immunizer known, is perhaps as highly differentiated a tissue. The need for further investigation is obvious, and this will be

TABLE 3

Tissues which will immunize against transplantable carcinoma are followed by the plus sign

Ectoderm	Entoderm
Fetal skin.....+	Liver.....+
Mamma.....+	
Lens.....-	All Layers
Brain.....-?	
	Embryo.....+
Mesoderm	
Spleen (containing blood).....+	Extraembryonic Ectoderm and Mesoderm
Blood.....+	
Testis.....+	
Kidney.....+	Placenta (washed free of blood)....+
Cartilage.....-	
Bone.....-	
Muscle.....-	
Lymph-node.....+	

undertaken as soon as conditions permit; indeed, it is even now under way. The present communication, therefore, is to be regarded as scarcely more than a preliminary report.

SUMMARY

Preliminary treatment with normal tissues containing but few cells, whether they be of ectodermal or mesodermal origin, fails to induce immunity to transplantable carcinomata. Muscle, also, though this is more cellular, is inactive, for some reason at present unknown.

Lymph-node, on the contrary, has the power to elicit a high resistance against transplantable carcinomata.

The mesodermal tissues investigated have no power to immunize against two connective tissue tumors employed, failing, like the skin, to protect against sarcoma.

REFERENCES

- (1) SCHÖNE: Münch. med. Woch., 1906, liii, 2519.
- (2) BORREL AND BRIDRÉ: Bull. de l'Inst. Past., 1907, v, 605. Ann. de l'Inst. Past., 1907, xxi, 769.
- (3) RUSSELL: Fifth Sci. Report, Imperial Cancer Research Fund, London, 1912, 23.
- (4) UHLENHUTH AND WEIDANZ: Arb. a. d. k. Gsndhtsamte., 1909, xxx, 443.
- (5) WOGLOM: Jour. Exper. Med., 1912, xvi, 629.
- (6) BULLOCK AND ROHDENBURG: Jour. Cancer Research, 1917, ii, 456.
- (7) HAALAND: Proc. Roy. Soc., Series B, 1909-10, lxxxii, 293; Lancet, 1910, i, 787.
- (8) BORREL AND BRIDRÉ: Cited by Bashford, Murray, and Haaland, Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 376.
- (9) TSURUMI: Jour. Path. and Bacteriol., 1915-16, xx, 76.

PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH

ELEVENTH ANNUAL MEETING

Held in Philadelphia, March 28, 1918

1. REPORT OF THE COUNCIL

The following members were present at this meeting: Dr. F. C. Wood, president; Dr. William H. Woglom, secretary and treasurer; Dr. James Ewing, Dr. H. Gideon Wells. Absent, Dr. Harvey R. Gaylord, and Dr. James B. Murphy.

The report of the treasurer, showing a balance on hand of \$275.19, was read and accepted.

Dr. E. R. Le Count, of Rush Medical College, Chicago, was elected as councillor to complete the term of the late Dr. Richard Weil, whose period in office would have expired in 1921.

Dr. Robert B. Greenough, of Boston, was elected to the Council to succeed Dr. James Ewing, automatically retired by the time limit.

The following officers were elected for the ensuing year: Dr. E. R. Le Count, president; Dr. H. Gideon Wells, vice-president; Dr. William H. Woglom, secretary and treasurer (re-elected).

The present members of the Council, with the years of their retirement, are:

Dr. Harvey R. Gaylord, 1919

Dr. H. Gideon Wells, 1920

Dr. E. R. Le Count, 1921

Dr. Robert B. Greenough, 1925

Dr. F. C. Wood, 1922

Dr. James B. Murphy, 1923

Dr. William H. Woglom, 1924

Dr. William H. Woglom was elected managing editor to succeed the late Dr. Richard Weil, and Dr. Frederick Prime was appointed associate editor. Dr. James Ewing was placed upon the Editorial Board, of which the other members were retained in office for the ensuing year. This board is now composed, therefore, as follows:

Dr. William H. Woglom,
Columbia University

Dr. Frederick Prime,
Columbia University

Dr. Joseph C. Bloodgood,
Johns Hopkins University

Dr. Leo Loeb,
Washington University

Dr. Ernest E. Tyzzer,
Harvard University

Dr. H. Gideon Wells,
University of Chicago

Dr. James Ewing
Cornell University

NEW MEMBERS

Honorary Member

Dr. Katsusaburo Yamagiwa, Imperial University, Tokyo, Japan

Active Members

Dr. Kenneth M. Lynch, Charleston, South Carolina

Dr. Angelo Roffo, Buenos Aires, Argentine Republic

Dr. Douglas Symmers, New York

Dr. F. W. Baeslack, Detroit, Mich., was advanced from associate to active membership.

The resignations of the following members were accepted: Dr. E. V. L. Brown, Dr. L. D. Bristol, Dr. G. N. Calkins, Dr. P. Rous, Dr. H. K. Oliver, Dr. P. Woolley, Dr. J. Bowen, Dr. H. Smith.

The secretary reported that he did not carry out the instructions of last year regarding the preparation of a printed list of subscribers to be sent to all members, with a request that they endeavor to secure new members, because on the day after the meeting the United States declared that a state of war existed with Germany. He therefore regarded it as unwise to incur the expenses incidental to a subscription campaign. In this he was sustained by the Council.

Dr. Wells reported that progress has been made in arranging a federation of our society with the American Association of Pathologists and Bacteriologists and the American Association of Immunologists.

In opening the morning session, Dr. Wood referred to the blow sustained by the Association in the loss of Dr. Richard Weil, who had been interested in the early progress of the society and had for some years served as Editor of the JOURNAL. He had regularly attended the meetings, had read papers and taken part in the discussions, and had been at one time President. He did what all would like to do—volunteered in the service of his country; and, while working at one of the camps in the South on the pneumonia question, was himself stricken with the disease and died after a brief illness.

It was moved and seconded that the Chair designate a committee to inscribe a memorial for transmission to Dr. Weil's family. The president accordingly appointed Dr. H. G. Wells and Dr. James Ewing.

2. THE LYMPH-NODES IN IMMUNE RATS

Dr. William H. Woglom (New York):

SUMMARY

When lymph-nodes from rats with progressively growing or receding tumors are compared with those from normal rats, it is found that except for some slight hyperplasia of the germinal centers in the lymph-nodes from the tumor-bearing animals there is no morphological differ-

ence. Whether this change is due to the presence of the tumor or not, it is impossible to say at present, nor can its significance be forecast. The investigation is in progress.

3. SOME LATE AND DISTANT EFFECTS OF RADIUM

Dr. James Ewing (New York):

SUMMARY

Ever since Dominici employed filtration of the soft rays of radium, this method has been universally used to secure the deeper curative effects of radium in the treatment of cancer. The method is generally very effective in its proper field, but there are certain limitations in the use of heavily filtered radium which it is important to recognize.

During the past few years I have encountered in operative and post-mortem material at the Memorial Hospital in New York, evidences of the late and distant effects of large doses of heavily filtered radium which are of importance in controlling the use of this method. That the application of a large amount, 500 mgm. to 1 gram of radium, filtered through lead and applied for twelve to twenty-four hours, at a distance of 5 to 10 cm. from the skin, is capable of causing definite regressive changes in deep seated tumors I have repeatedly observed. Among the tumors most susceptible to this action may be mentioned mediastinal lympho-sarcoma, carcinoma of the lung, and abdominal metastases of carcinoma of the testis. In the latter type of growth some very remarkable and gratifying results not infrequently follow the use of radium, but I have observed certain definite conditions caused by this tumor which can not be successfully met by radium. Thus in one very bulky metastasis, the tumor broke down rapidly; the growth was the seat of an extensive hemorrhage and the patient died, largely as a result of the loss of blood. A less vigorous application with slower effect might have resulted more favorably. In two other cases similar treatment was given without any effect upon the size of the tumor, and autopsies disclosed that these growths were of large size, firm and fibrous, encasing the great vessels and causing edema of the limbs. With this anatomical condition to deal with, it is quite impossible to expect radium to cause any great reduction in the size of a neoplasm. The most it could do would be to cause fibrosis and cicatrization which, under the circumstances, would only aggravate the constriction of the vessels. In a great many conditions a similar deposit of fibrous connective tissue prevents any marked visible effect of radium. In fact it may be stated, as a rule, that if diminution in size is to be taken as the indication of therapeutic success, this rule can be applied only to cellular, not to fibrous tumors.

In the case of osteogenic sarcoma, where there is often much fibrous, cartilaginous, osteoid, or osseous stroma, diminution in size cannot be expected as a sign of radium effect, and cessation of growth followed by a long period of gradual shrinkage is all that should be looked for.

In a bulky carcinoma of the lung, the radium pack caused extensive necrosis of most of the tumor, but an invasion of the spinal column destroyed a vertebral body and led to paraplegia. Protected by the bony tissue, the invading tumor cells appeared to be entirely unaffected by the radium.

In general it may be said that, in dealing with bulky deep seated tumors, the result will be strictly dependent upon the structure of the growth and its accessibility to radium, but that the clinical outcome will not always accord with the amount of influence exerted on the tumor. I feel disposed to recommend that such deep seated tumors be treated rather cautiously.

Arteriosclerosis is very frequently observed in the neighborhood of carcinoma of the skin or mucous membrane, and, indeed, arteriosclerosis has been urged by many as a fundamental predisposing condition to cancer in these tissues. Its probable existence should be taken into consideration in attempting to treat any well established carcinoma of a mucous membrane, especially in elderly subjects, by heavily filtered radium. For radium itself tends to produce a slow and progressive obliterating endarteritis, the effects of which may not be seen for weeks or months after the exposure. The results of such endarteritis may be late sloughing, or the production of an indolent ulcer which refuses to heal although most or all of the cancer tissue has been destroyed. In such cases, the use of small amounts of unfiltered radium, the radius of action of which is superficial and restricted, should be considered, and in general, I believe, preferred.

Many workers with heavily filtered radium have observed that with each repeated dose the regressive effects in the tumor are less and less definite. It has even appeared at times that the tumor tissue became very resistant to the radium, while the normal tissue, especially the stroma, became less resistant. In a case of basal cell carcinoma of the scalp, repeatedly excised and returning each time over a wider area, then treated with the x-ray and finally by repeated doses of filtered radium without satisfactory results, I found on the edges of the ulcer apparently living and well staining tumor cells, lying in a hyaline or necrosing connective tissue entirely devoid of circulation and quite incapable of any effort at healing. It is not possible in this case to say that the tumor cells were growing; the lesion was not advancing clinically, but it is certain that no important therapeutic result had been secured by the radium.

To what extent this reversal of the relative susceptibilities of tumor tissue and normal tissue occurs after the use of repeated doses of heavily filtered radium, I am unable to state, but I believe that it represents a definite limitation of repeated treatments by the gamma ray.

An important therapeutic use of heavily filtered radium which is probably inadequately recognized, but which has been pointed out by Levin for the x-ray, consists in the retardation of the rate of growth of the tumor cells and the transformation of an active malignant into a

slowly growing or quiescent and relatively benign tumor. In dealing with very advanced epidermoid carcinoma, or active adenocarcinoma which has advanced beyond the area which can effectively be reached by radium, most observers realize that the object of treatment should be palliative and not curative. Under such circumstances the use of heavily filtered radium over long periods at a distance from the skin may often bring the tumor to a standstill, relieve pain, reduce pressure, permit the patient to take on weight and prolong life without inflicting any perceivable injury or discomfort. It is in this field that radium has proved of inestimable value as a palliative, whereas more aggressive attempts to eradicate completely the disease, in those deplorable post-operative recurrences which are constantly being thrust upon the hands of the radium worker, often end disastrously.

Among the interesting results of this type which I have observed is one in which an originally active and cellular epidermoid carcinoma of the lip, metastatic in the lymph-nodes, had been transformed into a structure replacing the nodes and resembling a sebaceous cyst lined by normal skin. In another case, an epidermoid carcinoma that was invading the labial artery had been transformed into a structure resembling normal epidermis. Such transformations are occasionally seen to arise spontaneously in the course of epidermoid carcinoma, but they are very frequently seen in cases treated by radium.

It is a general experience in radium clinics that some cases of epidermoid carcinoma respond badly to radium treatment and after an initial inhibition of growth seem to progress very rapidly, even more rapidly than before, so that the question of an acceleration of growth has arisen. This explanation has been accepted by those inclined to do so. In several such cases, I have been able to demonstrate a satisfactory cause for the unfavorable course of the tumor and the rapid decline of the patient, consisting in the presence of active tuberculosis of the lungs and other organs, with tuberculous infection of the tumor; while in other cases, the patient was suffering from advanced tertiary syphilis of the organs and of the tissues and vessels in the vicinity of the tumor. While in these latter cases syphilis was suspected from the clinical signs and a positive Wassermann, the tuberculous complication in the former was overlooked in several instances.

These observations merely emphasize the fact that in treating carcinoma by means of radium, or of any other agent, we are not always dealing with a perfectly healthy body nor with an uncomplicated local tumor, and that a broad general clinical point of view must guide the therapist in the use of such a powerful agent as radium.

In a previous communication, based largely on the clinical work of my colleagues, I expressed the view that the constitutional action of large doses of heavily filtered radium was not a serious fault in its use and that such constitutional action, if it occurred, was largely referable to active necrosis of tumor tissue and to infection. Further experience confirms this opinion, since it is often shown that even rather large tumors may be rapidly absorbed without constitutional disturbance.

On the other hand, when the tumor is infected, especially in epidermoid carcinoma, the use of radium in any form may be followed by aggravation of the infection with spreading phlegmonous inflammation, erysipelas, pneumonia, or septicemia. Fortunately, these serious complications are rare, but the possibility of their occurrence must be kept in mind in any form of radium treatment of an advanced and infected malignant tumor.

Even this brief catalogue of some of the things which radium can not accomplish with advanced carcinoma, and especially with post-operative recurrences, inclines me more and more to reaffirm the position taken two years ago, that the most successful and, accordingly the proper field for radium, will be found in strictly localized and therefore operable carcinoma.

DISCUSSION

Dr. H. Gideon Wells (Chicago): Dr. Ewing suggested that arteriosclerosis follows the destruction of tissues on account of the diminished volume of blood passing through the vessels. This, in itself, might perhaps account for a great deal of the arteriosclerosis that is seen in such cases.

Dr. F. C. Wood (New York): Dr. Ewing has brought up many interesting points, for instance, alteration in the type of a tumor. I have been receiving material from a colleague who treats many tumors about the face, and find that I get for examination only those which have resisted a considerable quantity of radium. Basal-cell tumors which contain keratin seem able to resist radium, and I have been endeavoring to get material before the tumor is treated, to see whether radium kills cells of the basal type and spares squamous elements, or whether the basal cells differentiate into squamous cells under radium treatment; in other words, whether this is another example of the change of type discussed by Dr. Ewing.

Radium toxicity is also interesting. I have seen one or two cases of this condition, but without extensive changes in the tumor, or resorption, such as Dr. Ewing has observed. I have wondered whether it is not due, in some cases at least, to progressive destruction of the bone marrow, or to an alteration in the function of the liver, brought about by radiation which has escaped. We do not always remember, in employing radium, that more or less remote regions also come under its influence. The anemia which so often occurs in leukemic patients that are receiving treatments over the spleen, indicates the wide influence of radiation. The shrinkage that takes place in the spleen within a few days in these leukemic cases, brings up the question why these enormous splenic tumors should disappear without causing toxic symptoms. I have not seen a severe toxemia after radium in any case of myelogenous leukemia.

A third point in Dr. Ewing's remarks is the question of a development of resistance to radium under certain conditions. Dr. Frederick Prime and I are engaged in a series of experiments which we hope will show whether or not resistance supervenes in cases in which tumor cells are transplanted through a series of animals. We radiated a tumor, transplanted it, radiated it again, and transplanted it again, in order to see whether there was any difference in the number of takes and in the rate of growth. We found no difference. My theory is that after most of the carcinoma has been killed by radium, the few remaining cells at the periphery have sufficient nutrition to take care of themselves, being at a site where the vascular supply is abundant.

The lethal dose for cells *in vitro* is only half of that necessary *in vivo*. That is, to kill a cancer cell where there is no circulation requires about half the radiation requisite where the circulation is still in full force; whatever the products are that destroy the cells, they appear to be swept away by the circulation.

4. EFFECT OF *x*-RAY ON TUMORS

Dr. Francis C. Wood and Dr. Frederick Prime (New York):

SUMMARY

While much has been done with the *x*-ray of late, the lethal dose for tumor cells under what may be called the usual conditions employed in practical work has not yet been accurately determined. Nevertheless, such a lethal dose must be determined if *x*-rays are to be intelligently used in the treatment of internal malignant growths. The conditions of superficial treatment, on the other hand, are well enough known.

In order to determine this lethal dose, therefore, the following experiments were carried out.

As with previous experiments in which radium instead of *x*-ray was employed, the tumors used were a mouse sarcoma (Crocker Fund No 180) and a mouse carcinoma (Crocker Fund 11); mouse embryo kidney was also used. The material was removed under aseptic precautions and cut into small pieces, about 0.001 gram in size. These were moistened with a drop of Ringer's solution and put into hollow slides, which were then covered with cover-glasses and sealed.

About twelve minutes were required to produce an erythema reaction with the *x*-ray apparatus employed, at a distance of 9 inches from the anticathode of a Coolidge tube with 3 mm. of aluminium as a filter, when 5 milliamperes of current were used with an 8.5 inch spark gap. The tissue was treated in gradually increasing doses ranging from six to forty-five minutes.

Some of the treated tissue was then inoculated into mice and the rest was planted in plasma. The results were as follows:

Where sarcoma was inoculated at once into mice after treatment with *x-ray*, there was no difference in the number of takes or in the rate of growth between controls and treated tissue, until the tumor had been given *x-ray* for twenty-five minutes; from this point on, there was a decrease in the number of takes and the rate of growth, until at forty-five minutes there were practically no takes.

The tumor which had been radiumized and planted in plasma, however, showed most profuse growth even at the end of forty-five minutes. This growth was photographed for record and the plasma plants then inoculated into mice, where they grew in practically the same manner as did the tissue which had not been planted in plasma.

With mouse embryo kidney the same profuse growth was shown in tissue that had been given *x-ray* up to forty-five minutes, but no controls could be made by inoculating into animals.

Mouse carcinoma was subjected to the same treatment, with a slightly different result. There was never such profuse growth as was seen in the sarcoma and kidney tissues, and the time required to slow the rate of growth was less, since after an exposure of twenty-five minutes there was no growth after inoculating the tissue into mice, though there was still a slight *in vitro* growth when pieces of *x-rayed* tumor were planted in plasma.

Another series of experiments was undertaken, in which mice with tumors were treated with *x-ray*, after which the tumor was removed and inoculated. In these experiments the mice were put in a small box and treated for from ten to sixty minutes. At the end of forty minutes the transplanted tumors began to show some slowing, but even after being treated for sixty minutes there was good growth at the end of two weeks in about 50 per cent of the mice inoculated.

While these experiments are not yet in shape for final report, it is evident that about twice as much radiation is required to kill tumor cells *in vivo* as *in vitro*. In other words, seven to eight erythema doses must be applied at a single sitting to kill cancer cells.

DISCUSSION

Dr. Ewing: I should judge that the dose that Dr. Prime uses exceeds the dose that one attempts to employ in therapeutics for the treatment of a primary tumor, for the control of metastasis, or in postoperative radiation. If that be so, the results would indicate that any reasonable amount of *x-ray* treatment in these circumstances is without experimental basis; yet clinical experience shows pretty definitely that human tumors will regress rapidly, at times, after the application of a much smaller amount of *x-ray* than Dr. Prime is using, if my estimate of the dosage he employs be correct. In other words, the results of different investigators are diametrically opposite. Is there any explanation for this? I think there is one that should not be lost sight of. Dr. Prime is estimating the effect of rays on tumor cells only;

but these are not the only elements concerned in therapeutic experience. Surrounding cells in the body are also affected, as histological studies show. Thus in the regression of epidermoid carcinoma I have found tumor cells rapidly disappearing by a process of growth of granulation tissue; and I drew the conclusion that recession is the result of this proliferation of the stroma, and not of the radiation itself.

Again, the question of the amount of growth actually taking place *in vitro* must not be lost sight of. Whether the separation of cells from the main parent mass can be interpreted wholly as due to growth, or whether it be partly an expression of other physical changes in the parent mass, is, I think, open to question. The better test is the biological test: What is the result of transplanting the tumor cells back into the animal? If they grow, we are sure that they have not been killed; yet even this is not a complete duplicate of therapeutic experiments. Nevertheless, I welcome this type of investigation, and believe it important that the activities of the radium therapists be supported by this sort of experiment.

Dr. Prime: I think that perhaps Dr. Ewing does not realize how hard it is to duplicate therapeutic doses in the laboratory. The slides were exhibited in order to show that even forty-five minutes exposure does not kill. We obtained exactly the same result with radium.

Dr. Wood: This work is part of a general experiment, outlined to discover whether there is any difference between the cancer cell and the normal cell; and if there be, to determine what it is. The reason that tissue cultures were employed is that the most convenient method at present of demonstrating life is to show that the cell will grow; vital stains, also, may occasionally be of service. Furthermore, tissue cultures permit a study of the effect of radiation on cells entirely isolated from the body as Dr. Ewing has pointed out, so that the conditions of the experiment are as simple as they can be made. We have endeavored, in all other respects, to reproduce the conditions under which the clinician employs the x-ray. The filter ordinarily chosen by the practitioner is one that will permit an attack of the deeper tissue, and we accordingly used one of three millimeters, such as he usually employs.

Our figures were obtained from tumors of the highest virulence. A cell from a basal-cell epithelioma is probably twenty times as susceptible to x-ray as one from a mouse tumor, but I do not doubt that there is a type of human neoplasm that is as resistant as any mouse tumor. We must conclude that to kill a tumor cell just beneath the skin it is necessary to use eight times the erythema dose at a single sitting. To kill the ovarian follicles requires five hours' exposure, and a tumor cell is still more resistant. The necessity for this enormous dose is due to the increased distance from the tube, and to the absorptive power of the tissue, 8 cm. of which remove about two-thirds of the energy of the rays employed. Obviously such doses are clinically impossible. We are trying to work out a method of treatment that the

practitioner can apply, and to do this we must study the various susceptibilities of human tumors. The curious thing is that these experiments show the cancer cell to be more resistant than the skin, since six or eight erythema doses are required to destroy it.

The dividing cell is known to be three times as sensitive to radium as others, and variability in the number of takes is perhaps due to the stage of division that the majority of cells in a tumor are in; in one growth, most of them may have just passed through mitosis, while in another the majority may be in a pre-mitotic or mitotic phase.

The percentage of takes shows fluctuation in the material, it is true; but one single take proves that that tumor would have recurred in a human being.

Dr. W. T. Bowie (Boston): It might be possible to get a differentiation that one could see under the microscope, by raising the temperature of the cells above normal. In some experiments that I expect to describe more fully this afternoon, I found that I can radiate paramaecium with ultraviolet light without producing any morphological change, until the temperature is raised to 24°C. At this point, the protoplasm promptly cytolyses.

Dr. William Duane (Boston): May I ask what the temperature was?

Dr. Prime: 37° in the incubator.

Dr. Duane: There is a surprising difference in x -ray radiation when the temperature is changed. Penetration increases also with the voltage; doubling the voltage produces eight times the penetration. Measurement of the voltage by the spark-gap is not very accurate for it gives no information about the intermediate voltage. It may be that the intermediate voltage between these points is very small. This should be taken into account when experiments made with one machine in one place are compared with other experiments made with a different machine in another place.

Dr. Wood: That is true. But we found accurate measurement a waste of time so far as our purpose was concerned, which was merely to get practicable results for the use of the clinician. I fully realize, however, that there is a wide difference in apparatus, for one of my assistants has a duplicate machine, installed within a few weeks of ours at the Crocker Fund, and actually adjusted by the same man; yet the two give entirely different results, as determined by the photographic or the pastille method.

Dr. Ewing: In 1913, in Paris, Keating-Hart was working with an x -ray apparatus with which he said he was curing advanced carcinoma of the skin. He said, "You cannot cure it with the x -ray, unless you

raise the temperature;" and he did so, putting one electrode under the patient's body and connecting it with aluminum foil, and raising the temperature until the patient cried out from the heat.

5. THE COMPARATIVE PATHOLOGY OF TUMORS OF THE TESTIS

Dr. Maud Slye, Miss Harriet F. Holmes, and Dr. H. Gideon Wells (Chicago):

SUMMARY

New growths of the testis are common in horses and dogs, exhibiting quite the same characteristics as corresponding tumors in man. But they seem to be very rare in all other species, and no mention of such a tumor in the mouse can be found in the literature. In the Slye stock, among 19,000 mice dying natural deaths and examined post mortem, about one-half of which were males, 28 instances of primary growth of the testis were found. Most of these resembled in all essential features the neoplasms that arise in the testis of man and other animals, consisting of cells closely resembling the epithelium of the seminiferous tubules and often arranged in an alveolar structure. Despite great vascularity and a distinctly atypical structure, no remote metastasis was observed, although in one case a series of six contiguous independent nodules was formed, and one had tumors in both testes. Two of the cases seemed to be true spindle-cell sarcoma, one arising at the site of a wound. Three of the typical "orchidoblastomas" also followed trauma. No evidence could be obtained that any of these tumors had arisen in a teratomatous growth, and no cases of teratoma have been observed.

One case of spindle-cell sarcoma of the seminal vesicle of a mouse is described, apparently the second case of a tumor of this organ reported as occurring in a lower animal.

Two cases of primary spontaneous tumor of the testis in dogs are described.

With the exception of one sarcoma, all the 28 new growths of the mouse testis occurred in the members of a single strain of mice and its hybrid derivatives, thus substantiating the statement that heredity influences the incidence of tumor development in different organs or tissues. This fact also probably explains the absence of any recorded cases of tumor of the testis in mice from other laboratories.

DISCUSSION

Dr. Woglom: The paper of Dr. Wells has great interest for me, because for the past three years or so I have been trying to produce tumors in mice. There are three probable causes of cancer so far known—age, hereditary predisposition, and perhaps some form of chronic irritation. Since in a mouse with a spontaneous new growth

two of these are already present, only such mice have been employed. Yet although various sorts of irritation have been applied, including tar, as recently described by two Japanese investigators, no tumor has been produced. Some of Dr. Wells' growths followed trauma, and it occurs to me that these mice may have lived longer than mine, none of which survived much more than five or six months, since they are already middle-aged when they reach the laboratory. I should be greatly obliged to Dr. Wells, if he would tell me how long an interval elapses between the injury and the tumor.

Dr. Leo Loeb (St. Louis): Several years ago we began some experiments in which we applied long-continued stimulation of various tissues to certain mice in which we had previously established a considerable predisposition to cancer. Both theoretical consideration and well-known facts had suggested to us that hereditary predisposition and external or internal stimuli (internal secretions) are each of importance in the etiology of cancer—a view that we have expressed on various occasions. We applied tar to the skin of our mice, or we caused chronic ulceration in other ways; in some cases, again, we pulled a thread through the mammary gland. But so far our experiments have all been negative, no cancer having been produced in this way.

Dr. Wells: I cannot answer Dr. Woglom's question how long after the trauma the tumor developed, because Miss Slye has the records. The system on which we have done the work was to keep the one end as far from the other as possible. I did not know the heredity, and she did not know the diagnosis, until we brought our parts of the work together.

6. THE EFFECT OF HEAT UPON TUMOR TISSUE

Dr. Holland N. Stevenson (New York):

SUMMARY

The effect of heat upon tumor tissue has been studied by several investigators and some interesting points in regard to the general biological properties of tumor cells have been determined. The work of Loeb stands out prominently. This author, using large numbers of animals, demonstrated the progressive inhibition of the growth power of a mouse carcinoma when kept at 44°C. for from five to sixty minutes. He found that the latent period of growth and the number of regressing tumors increased with the time of exposure to the heat, and demonstrated that these effects were not transferred, in any striking degree, to subsequent generations. Lambert later showed that tumor was more susceptible to heat than adult connective tissue, when both were growing *in vitro*. Haaland was able to eliminate the carcinomatous portion of a carcinosarcoma by exposure to heat, an indication that the carcinoma cells were more susceptible to heat than sarcoma

cells. Various other investigators (Clowes, Clowes and Baeslack, and Michaelis) have also described the effect of heat upon tumors.

The object of the experiments here reported was to establish accurately the lethal points for tumor exposed to a series of temperatures for varying lengths of time, so that the death points could be used as a basis for further work with normal tissues. Another object was to determine, if possible, the curve that these points would make if plotted according to the factors of temperature and time of exposure. It was also of interest to determine whether or not carcinoma cells were actually much more susceptible to the influence of heat than sarcoma or normal epithelial and connective tissue cells.

The tumors used were the Crocker mouse carcinoma 11, and mouse sarcoma 180. These have a growth rate approximately the same, although the latter grows a little more rapidly and has a little higher percentage of takes. The tumors were cut into small pieces (0.002 grams), of which 24 were placed each in a small test tube containing about 1 cc. of Ringer's solution. The tubes were then placed in a large Wassermann water bath, of such size that temperature fluctuations due to radiation were reduced to a minimum; control pieces were placed in the ice-box. The temperature of the water bath was registered on thermometers reading to 0.01°C . and checked by delicate certified thermometers. At definite periods, readings of the thermometer were recorded and a tube containing a fragment of tumor was removed; the tumor was then cooled and inoculated into 24 mice, control tumor from the ice-box being inoculated at the same time.

The results showed that the death points of the tumors fall upon a curve which must be made rather broad in order to take in all the deviations encountered. These deviations must be due to different biological conditions of cells in the same tumor particle which it was impossible to control (e.g., mitosis, etc.), and to the different tumors used, rather than to temperature or other physical variations.

At 39.5 to 40.5°C ., the sarcoma stopped growing after two and one-half hours' exposure. The carcinoma showed no growth after two hours, except that one stray tumor developed at four hours.

At 42.5° the sarcoma stopped growing after forty-five minutes' exposure, while at 44° it did not grow after one hour, except that two stray tumors developed after two hours' exposure. The carcinoma was found to stop growing after forty-five minutes at 44° .

At 47.75° to 48.25° no growth of either sarcoma or carcinoma occurred after fifteen minutes' exposure.

A review of these results shows that the curve for each tumor is broad, and not limited to such a definite line as a rectangular hyperbola. This is especially indicated by the occurrence of tumors (sarcoma) at 44° after one hour exposure when at a lower temperature (42.5°) they do not grow after less exposure, as well as by the development of tumors from one or two grafts after an exposure long enough to kill tumor cells in other cases.

Neglecting those tumors which appeared after a time of exposure far beyond the period when the other portion of the tissue had been killed, and considering as the death point the time of exposure at a certain temperature when no tumors developed, it was found that through these points a curve could be passed that would be approximately at a minimal distance from them. This curve was a simple hyperbola, plotted according to the formula $XY = K$, with K in this instance arbitrarily equal to 15. If the factor K be made equal to 8.8, and again to 20, two curves are obtained on or within which all the determined death points fall.

These points were plotted on the rectangular coordinates Xy , X being the time of exposure and y the temperature. The zero of temperature was taken as 39°C . the approximate body temperature of the animal, for it was considered that at this temperature the tumor tissue would live indefinitely; or if $y = 0$ then $x = \infty$.

From the results obtained, no difference between sarcoma or carcinoma in their susceptibility to heat can be made out. The experiments are to be continued to include normal epithelium and connective tissue.

DISCUSSION

Dr. Wells: I should like to ask Dr. Stevenson whether he attempted to find, in the few cases where tumors grew after exposure to the normal death point, whether any difference in thermo-resistance appeared in subsequent generations.

Dr. G. H. A. Cloues (Buffalo): I should like to ask Dr. Stevenson whether there was any evidence of a stimulation of growth. We observed this in a few cases, but were unable to reproduce the phenomenon in a later attempt.

Dr. Loeb: I would ask Dr. Stevenson whether there has been noticeable, in any case, an increase in the duration of the latent period after the transplantation of heated tumors.

Dr. Stevenson: In reply to Dr. Wells, I would say that no attempt has yet been made to establish a thermo-resistant tumor.

Neither retardation nor stimulation could be demonstrated.

With reference to an increase in the duration of the latent period, it may be said that this was not constant enough to be of any value. The number of instances in which the latent period was increased was not sufficient to indicate anything definite.

Dr. Wood: Here, again, I think that fluctuations mean differences in the composition of the biological material. The number of cells that have divided is uneven in samples obtained by selecting pieces of a tumor.

7. FURTHER STUDIES ON THE IMPORTANCE OF THE LYMPHOCYTE IN CANCER IMMUNITY

Dr. M. J. Sittenfield (New York):

SUMMARY

These experiments are supplementary to those reported before this Association last year, and corroborate them entirely.

The experiments of Murphy and Morton with mice were repeated with the Flexner-Jobling rat carcinoma, but no relation between the lymphocyte and immunity to transplanted tumors could be demonstrated.

DISCUSSION

Dr. Wells: We have frequently observed that spontaneous tumors develop in mice with lymphatic or myelogenous leukemia, as they do in other mice; and that even where there is a prodigious increase in the lymphocytes, the tumor is entirely unaffected.

Dr. Loeb: Might it not be that two factors are necessary for the action of the lymphocyte? Lymphocytes must be present in sufficient numbers, and the path to the tumor must be free. The tissue must exert an attraction on the lymphocytes, and it is this latter factor that is especially important.

In the case of homoiotransplantation, lymphocytes and connective tissue are very important factors in the destruction of tissues, but this applies only to certain varieties. With others, and in heterotransplantation, the lymphocyte plays a minor part, and the incompatibility of the body fluids suffices to destroy the graft.

Dr. H. T. Marshall (Virginia): In cancers and other pathological conditions it is often difficult to escape the impression that lymphocytes are in some way involved in the reaction to the pathological condition. In a case of lymphadenoma which I hope to report tomorrow there are certain appearances which, it seems to me, are most satisfactorily accounted for on the assumption that the lymphocytes are engaged in some form of protective reaction.

Dr. Wood: An interesting fact, from the morphological point of view, is the extreme facility with which carcinoma grows in the lymph-nodes in the human body. Again, we have at the Crocker Fund a guinea-pig sarcoma which takes in but few animals and grows very slowly. According to the hypothesis advanced by some investigators, reduction of the lymphocytes would facilitate the growth of this tumor; yet it exerted no effect at all. Finally, we have inoculated a leukemic mouse with a mouse tumor, and found that it grew as well as in a normal one.

Dr. Loeb: The great significance of the lymphocytes in the destruction of grafted tissues sometimes comes out very strikingly after the transplantation of thyroid from mother to child or from sister to brother. In such cases, transplants are often so perfectly preserved as almost to resemble autotransplants; but in most cases lymphocytes gradually begin to appear in large masses and to destroy the transplanted thyroid. We have every reason to assume that without the appearance of lymphocytes the tissue would have remained alive much longer.

Dr. Wood: How can you tell whether the lymphocytes appear as scavengers after the tissue has died, or whether they themselves destroy?

Dr. Loeb: Lymphocytes appear among acini that look perfectly well preserved, resembling those in a normal thyroid that has not been transplanted. Indeed the graft which is thus attacked often shows mitotic proliferation. The lymphocytes then actually invade these acini, and overwhelm and destroy them, the destruction occurring first where the lymphocytes appear first in largest number—that is, around the larger vessels. In heterotransplantation, tissues perish without the intervention of lymphocytes. Unstriated muscle dies sooner or later after homoiotransplantation, but lymphocytes are not often attracted in large number. Dying tissue does not, as such, attract lymphocytes; but certain metabolically abnormal tissue products, such as develop in epidermis and certain glands after homoiotransplantation, have this effect. These metabolic abnormalities, as such, would be compatible with a longer duration of life, were it not for the activity of the lymphocyte (and the connective tissues).

Dr. Wells: Surgeons say that the best place to transplant thyroid is in the bone-marrow and the spleen.

Dr. Loeb: That does not contradict what I have been saying; the essential factor is that there be some product in the cells which attracts the lymphocytes. The attraction is exerted upon the lymphocyte under certain conditions by the products of tissue metabolism.

Dr. Wells: On that account, it must be primary in the cell.

Dr. Loeb: Yes; it is the two factors combined. The stimulus plus the cell. If either be missing, the result is not forthcoming. However, there can be no doubt (and in this respect I agree with the opinion expressed here, which I myself have upheld on previous occasions) that lymphocytes play a part in destruction of tissue and tumor grafts only under certain conditions. Other factors are in play beyond question.

Dr. Ewing: I wish to speak of a matter which was mentioned last year in the discussion; I refer to the experience of the general pathologist at the autopsy table. I have long since given up trying to demonstrate any relation between the activity of the lymphoid apparatus in the human body and the growth of cancer, and I believe that it is impossible to do so. Actively growing tumors are found in young subjects with lymphoid tissue in great abundance; on the other hand, widespread neoplasia involves the bodies of elderly subjects, in whom the lymphoid apparatus is deficient. The same observation applies to the size of the spleen; I have been quite unable to discover any relation whatsoever between the rapidity of growth and metastasizing power of different tumors, and the size of the spleen. Hence I do not believe that any information can be drawn from postmortem experience to favor the theory that any part of tumor immunity is due to the lymphatic apparatus.

Dr. Sittenfeld: So many have defended me that very little remains to be said, except, perhaps, to supplement their statements by recalling the fact that malignant lymphomata occur in lymphoid tissue only

S. EXPERIMENTAL "CARCINOMATA" OF ANIMALS AND THEIR RELATION TO TRUE MALIGNANT TUMORS

Dr. F. D. Bullock and Dr. G. L. Rohdenburg (New York):

SUMMARY

None of the publications describing the experimental production of new growths can be accepted without reserve. Against them are the facts that the tumors are usually said to have been produced in young animals as readily as in old; that they generally do not continue their growth after the irritant has been discontinued; that they are not transplantable; and that no really reliable evidence of metastasis has been offered. The lesions were probably hyperregenerative processes.

A full description of a long series of unsuccessful attempts to produce tumors will be published at some future time.

DISCUSSION

Dr. Wells: I had recently the opportunity of visiting Professor Yamagiwa, and spent a large part of the day in going over the material at his laboratory. It is true that many of the simple regenerative lesions described here to-day appear in his material, but I saw specimens in which proliferation had continued after the application of tar had been stopped, causing widespread ulceration and reproducing histologically as perfect a carcinoma as I have ever seen; there were even metastases in the lymph-nodes, one of them 2 mm. in diameter. In some cases in which the irritant had been removed, ulceration had

continued for months, progressively destroying the tissues. After examining his material, I could see no reason to doubt that squamous-cell epithelioma had been produced by protracted chemical irritation.

I might add that a gastric cancer in a mouse, which I described at last year's meeting, was associated with a hair ball, and thus forms an interesting parallel to those experiments of Dr. Rohdenburg in which he irritated the gastric mucosa by introducing spinous balls into the stomach.

Dr. Loeb: I agree with Dr. Rohdenburg that a critical attitude regarding purely morphological criteria is necessary, where questions of the transformation of normal into cancerous tissue are concerned. However, there can be no doubt that such a transformation does certainly occur under the influence of long-continued stimulation; for instance, in those numerous cases in which a Roentgen ray dermatitis becomes gradually transformed into cancer. But the important point would be to determine when the biological change takes place; purely morphological criteria are often indecisive.

Dr. Ewing: It seems to me that between hyperregenerative overgrowth and true neoplastic hyperplasia, there is a distinction without any difference. I myself would conclude, not only from previous work but also from the startling results that Dr. Rohdenburg has presented here to-day, that he is describing lesions similar to or identical with the early stages of developing carcinoma in man. If he is going to assume that there is a distinction, he must have something more definite than mere position of cells in the tissues to support his contention. Dr. Loeb has referred to the fact that x-ray burns often become carcinomatous; yet these closely resemble the lesions just exhibited by Dr. Rohdenburg. The altered cells are strictly confined within the malpighian layer, and there is not a hint of danger to the patient. Nevertheless, and almost without exception, these cases of chronic x-ray dermatitis suddenly develop carcinoma. When the cells do break loose, progress is rapid.

I am impressed by the fact that islands of epidermization may be found in the lungs of rats; the matter was emphasized at Paris in 1913. At the same time, however, I cannot see that even though such a lesion is known to occur independently of carcinoma, we are justified in discarding the lesions which Fibiger has described.

While it seems wise to me, on the whole, not to be too hasty in drawing conclusions from purely histological data, I believe, nevertheless, that in the complete series of transitional stages which have been discussed here this morning, we are dealing with the beginnings of carcinoma.

Dr. Wood: It seems to me that Dr. Ewing has put his finger on the question; and also that the difference between true carcinoma and Dr. Rohdenburg's lesions, which he judged to be mere proliferations be-

cause they disappeared when the irritant was removed, is what brings us here this morning. Why a cell, whatever its position or morphology, should suddenly do something that a normal cell does not do is the problem upon which we are working.

Dr. Rohdenburg: I am, of course, at a disadvantage in having seen only the illustrations of Yamagiwa and Ichikawa, and not their original preparations. I agree with much that Dr. Ewing has said, and merely desired to point out the frequent danger of diagnosing malignancy solely on the ground of histology.

In order to produce a tumor it is probable that beside the irritant, some sort of tissue predisposition, as well as age, is required; and I am convinced that too little attention has been paid by experimentalists in the past to the factor last named.

Dr. Ewing: Is it not true that tumors in the lower animals are slow to metastasize—that the tissues of lower animals are not so favorable for metastasis as those of the human being? If so, we have no right to demand metastasis to the same extent in the lower animals that we do in man.

Dr. Wood: I believe that this is due merely to anatomical conditions. The lymphatic system in the lower animals is, perhaps, not so well developed, and that may be why metastasis in the lymph-nodes is rather uncommon in mice.

Dr. Wells: I used to accept the anatomical explanation for absence of metastasis until there came under my observation a strain of mice which often developed carcinoma of the liver. When we came to cast up the cases of metastatic tumors in the liver, we were astonished to find that nearly all of them occurred in this strain. It seemed obvious that the location of metastases was not, therefore, so much a matter of anatomy as had been supposed, but that some sort of tissue predisposition might be involved. We know, too, that the lungs of patients with gastric carcinoma are often full of emboli in all stages of healing, and we have good evidence of emboli passing through the lungs without settling there. And only recently I saw at autopsy a case of extensive carcinoma of the thyroid gland, with both adrenals, both mammary glands, and both ovaries extensively involved by metastasis. Such findings as these cannot be explained on an anatomical basis.

Dr. Loeb: I have had occasion previously to indicate that there is a great deal of evidence in favor of the conclusion that while both hereditary predisposition and external or internal stimulus are important for the origin of cancer, a certain reciprocal relation between these two sets of factors exists. If there be a powerful stimulus the amount of predisposition requisite is less; and it is very probable that in certain cases in which the stimulus has been unusually potent predisposition be-

comes almost or quite negligible. Thus it has come about that many roentgenologists who, in earlier years, did not know how to protect themselves, developed cancer; we cannot assume that they all happened to be predisposed. It is similar with the factor of age, which is important in animals as well as in man. Among cattle, for instance, cancer usually attacks old individuals, as I pointed out in 1899; yet old age is not an absolutely necessary factor. Malignant tumors may occur in young persons, and even in children; and in mice cancer of the mammary gland appears not rarely in relatively young animals, only six or seven months old. Age is therefore probably not a direct factor, and may merely be of significance in that it permits stimulation to extend over a long period of time. How far structural senile changes, as such, predispose to cancer, is unknown.

Dr. Wells: So far as the spontaneous tumors of mice are concerned, the age curve corresponds exactly with that in man. New growths appear in the human subject with increasing frequency from the twentieth year onward, and in mice at a corresponding period, i.e., from six months onward. I do not see, therefore, how age can be dismissed as a factor of no importance.

Dr. Loeb: I did not mean to say that age and predisposition are not important; merely that they do not seem to be indispensable. Cancer may appear in relatively young persons, if irritation has been applied for a sufficiently long period.

Dr. Rohdenburg: Regarding the length of time that it is necessary for an irritant to act, I would say that we have been investigating a tape-worm that appears to be largely responsible for sarcoma of the liver in rats. We have found that this grows at the rate of 0.5 cm. a month, and that the length of those associated with sarcomata proves they had been present for a year. This is a long period in a rat's life, as compared with that of a human being.

Dr. Clowes: There has been a great deal of discussion of the question of border-line cases, and I should like to know whether the speakers have made use of a simple method that has recently been devised for determining the permeability of the tissues concerned. We know that various toxic agents and chemicals can induce an increase in the permeability of sea-urchin's eggs, etc. If the damage be carried only to a certain point, recovery may be brought about by changing the environment; but if carried beyond this point, the damage cannot be recovered from. A similar experiment might be carried out on human or animal material, giving the investigator something definite and concrete to lay hold of.

Mr. M. C. Marsh (Buffalo): We have seen at the State Laboratory in Buffalo one female mouse with a typical mammary tumor arising at

the age of three months. The other mice of the strain, which has a high incidence, produce tumors at the usual time, after the age of six months.

Dr. Wood: One point was brought up by Dr. Wells to which I might contribute a few notes; I refer to the susceptibility of organs to metastasis. There has been a great deal of discussion on the inadvisability of removing a piece of tumor for purposes of diagnosis and there are, in fact, two schools. One prefers to excise a fragment for diagnosis, if necessary, while the other would extirpate a tumor on the clinical evidence alone, believing that in doubtful cases it is better for the patient that the growth be removed, even though it should ultimately prove not to have been malignant. The discussion has been rather acidulous, especially in New York City, where there has appeared a series of editorials on the subject. Dr. Bloodgood, of Baltimore, chief opponent of excision for diagnosis, says that he has no record of cure in any case where a portion was removed for diagnostic purposes. On the other hand, those tumors that I have seen cured at St. Luke's Hospital, in New York, have been the very ones from which a fragment had been extirpated for diagnosis.

We have carried out some experiments with the Flexner carcinoma on 228 rats. We found no more metastases in rats that had had pieces removed from their tumors than we found in the controls.

We were surprised to find, however, that there was a distinct difference (as high as 50 per cent) in the percentage of lung metastases in rats from three different dealers. This illustrates two points; First, the remarks of Dr. Wells concerning organ liability to metastasis. Secondly, it explains the frequent differences in the percentage of metastases reported by different investigators who have worked with this tumor.

Dr. Ewing: This is an important question, and I am glad that it has been brought up in this society, supported by experimental data. The problem has been attacked by clinicians, though with a great variation of opinion. Dr. Park and I are acutely interested in the matter, because I was responsible to a considerable degree for the introduction of a diagnostic service by the Health Department of New York City. I believe thoroughly in the wisdom of that move, under proper restrictions. My main objection to the conclusions of clinical observers is the one mentioned by Dr. Wood: they have drawn sweeping conclusions from limited personal experience, and under conditions which I believe to be wholly unscientific. Thus, they recommend almost without exception a cauterization of the wound made in removing the tissue. But I doubt whether the considerable approval with which the clinicians view this prophylactic procedure can be endorsed from the laboratory standpoint. I myself think that it is an undesirable addition to the damage already done to the tissue by the scalpel, and I would suggest to Dr. Wood, if I may, that it would be a useful

enlargement of his experiment to investigate the effect of this additional damage. I believe that after a clean incision into a tumor, without pressure or trauma, there is very little chance of spreading the disease, whereas the cautery produces a notable hyperemia over a wide area which almost certainly would increase the growth of the tumor itself, and, as I visualize the situation histologically, would increase the tendency to loosen the tumor cells.

One has to admit that the excision of a portion of any tumor for microscopic diagnosis is, after all, a confession of ignorance, and we deplore the fact that it is often impossible to determine the nature of a tumor in any other way; hence our procedure must be determined entirely by the danger to the patient brought about by diagnostic excision, on the one hand, and the greater danger of a wrong diagnosis on the other. We may be able, at some time in the future, to diagnose cancer without histological examination, but until that time comes, I, for one, would rather have my tongue cut out by someone who had proved that a cancer was present, than by one who merely thought that it might be. Especially when it is a matter of life and death, is it important that the surgeon should know what he is about, even at the risk of producing metastasis. I have known three instances in which a leg was amputated for what afterward proved to be a syphilitic process. Nevertheless, I am not an advocate of the indiscriminate and unlimited extirpation of fragments from all tumors, and I should be glad to hear the opinions of other members of the Association, particularly as to the advisability of cauterization after diagnostic excision.

Dr. B. F. Schreiner (Buffalo): During the past three or four years I have had approximately five or six hundred cases of cancer referred for diagnosis and treatment. I invariably excise a piece of the tumor, but never follow this procedure by cauterization of any kind. I have never seen any harm follow, so far as I can judge, though it is almost a routine procedure in our dispensary to remove a piece for diagnosis if there be any question as to the exact diagnosis.

Dr. Wood: We have a surgeon with us, and should like to have Dr. Semken discuss the subject from the surgical point of view.

Dr. George H. Semken (New York): I appreciate very much the opportunity to enter into this discussion, though I feel that I must adopt an attitude opposite to that taken by Dr. Ewing. In the first place, incision is supposed to increase malignancy or to produce metastasis, the former by reason of pressure or other mishandling, the latter by opening the lymph-spaces. Practically, it would not make much difference to the surgeon if malignancy were increased, because not much could occur in ten days. But it would make a difference, if there were cancer cells in the lymph-channels; and it is impossible to carry a knife through a tumor without opening some of the blood- and

lymph-spaces, so that the cancer cell is provided with a much readier access to them than under the ordinary conditions of growth.

I have seen a great deal of harm brought about by diagnostic excision. If a patient had but one, and were then operated on immediately, it would not be so bad; but many patients go from one physician to another, and I have seen those who had had seven or eight diagnostic excisions performed. I have not followed my own cases to their end results, so that they are not strictly comparable with Dr. Bloodgood's material. Nevertheless, I can hardly think that he is justified in his statement that all cases in which biopsy has been practiced end fatally for that reason. I think it would be best to restrict diagnostic excision to those cases in which it is imperative, and to warn the patient of the danger of submitting to more than one.

Dr. Wells: My own position is, I think, similar to that of Dr. Ewing; so long as we are not sure that we are not assisting in the spread of a tumor, we should limit biopsy to uncertain cases. The question of the site involved must also be taken into account; thus I am much more inclined to recommend complete removal of a tumor from the breast, where the operation does not cause serious trouble, without waiting for examination of an excised piece of tumor, than I am in the case of a tumor of the tongue or of the larynx.

9. (a) THE EFFECT OF CONTINUED INBREEDING ON THE CANCER RATE IN MICE

(b) THE CANCER RATE IN HYBRID STRAINS

Dr. Leo Loeb (St. Louis) and A. E. C. Lathrop. Read by Dr. Loeb.

SUMMARY

(a) In these investigations it was our aim to determine the effect which continued inbreeding exerts on tumor rate and tumor age in the various strains of mice which have been under our observation for a considerable number of years, with a view of analyzing the influence of heredity and of external factors in the origin of tumors.

We found that while in the majority of strains the differences in tumor rate and tumor age remained constant in the successive generations, in others certain variations became noticeable as a result of the continued propagation of these strains. In a few cases an increase in the tumor rate occurred in the later generations, but in the large majority of cases in which a change was observed there was a tendency towards a decrease in the tumor rate which in some instances was very marked.

These changes in the tumor rate concomitant with continued inbreeding seem to depend on the following two factors: (1) As a result of long-continued inbreeding in mice certain characteristics of a strain

may change, the strain becoming less prolific and less vigorous. These changes may be accompanied by a lowering in the tumor rate. This was especially evident in the case of the "No. 8" strain which had been inbred through the largest number of generations and through a considerable number of years. (2) In other cases it could be shown that in the course of continued propagation certain families, which were more resistant to certain diseases or which were naturally more prolific or otherwise favored by accidental conditions began numerically to preponderate in later generations. In some cases such families or substrains differed in their tumor rate from the main strain and thus as a result of selection within a strain differences in the tumor rate appeared in the course of continued inbreeding.

Our further investigations confirmed and still further emphasized our previous conclusion that on the whole in the mice with a higher tumor rate the tumors appear at a relatively earlier age than in those strains in which the tumor rate is lower.

In our experiments continued inbreeding was followed in certain strains by a decrease in prolificacy and vigor and the tumor rate varied correspondingly.

We did not inquire whether or not through selective mating a deteriorating effect of continued inbreeding could have been avoided. Our results in no wise depend on the possibility of inbreeding a strain through many generations without experiencing a weakening effect of the inbreeding.

SUMMARY

(b) 1. In order to test our previous conclusions concerning the tumor rate in hybrid strains, we carried out additional hybridizations for which we selected strains which differed markedly in their tumor rate and which had been followed through a number of generations and found constant in their behavior.

As control experiments, hybridizations between strains or families of a similar (either high, medium, or low) tumor rate served. In these cases the offspring showed a tumor rate similar to that of the parents.

2. In selecting for hybridization various groups of "Cream" mice representing a very low tumor strain, and "English Sable" mice representing a high tumor strain, we obtained in the large majority of cases hybrid strains with a tumor rate intermediate between that of both parent strains. In a few cases it approached somewhat the high tumor strain of the English, and in a few other cases the low tumor rate of the cream.

3. In several hybrids between the high tumor strain "English" and the low tumor strain "Silver," which latter was split off from the English, the high tumor rate of the English prevailed. In a number of cases the mice which served for hybridization were followed throughout their life and found to behave typically as to their tumor rate.

4. If we omit the strains in which both parents had a similar tumor rate, we found, altogether, in 24 hybrids, the higher tumor rate to be dominant. In 17 of these the mother strain dominated and in 7 the father strain. In 19 hybrid strains the tumor rate was intermediate. And in 10 strains the lower tumor rate dominated. In 8 of these the mother strain prevailed and in 2 the father strain. The low tumor rate was therefore dominant in approximately 18 per cent of the strains.

5. There does not therefore seem to be a fixed rule as to dominance in the tumor rate. In a considerable number of cases, and especially in well analyzed cases, the result was intermediate.

6. Altogether, in 25 of our hybrid strains the mother strain, and in 9 the father strain prevailed. In 19 strains the result was intermediate. We conclude that either father or mother strain may dominate and that the tumor rate is not in the strict sense of the term a sex-linked character. But the fact that the mother strain prevailed in a much larger number of our cases than the father strain, and that several times (but not in all cases) in reciprocal crosses the hybrids followed the tumor rate of the mother strain, suggests the possibility that, as far as the hereditary transmission of mammary cancer in mice is concerned, the mother may be more potent than the father, and that perhaps under certain quantitatively varying conditions the mother strain may dominate over the father strain. This statement is merely a tentative conclusion at the present time, and needs further investigation before we can consider it as proved.

7. The results of these investigations confirm our previous conclusion, that in the majority of the crosses which we observed the cancer rate is either intermediate between those of father and mother strain, or that it follows the tumor rate of the parent with the higher rate; and that only in a relatively small number of cases does it follow that of the parent strain with the lower tumor rate. On the whole, the heredity of cancer rate and cancer age follows the blending type of hereditary transmission.

8. While there is a distinct relationship between high tumor rate and early cancer age, our observations make it probable that cancer rate and cancer age are to some extent independent of each other.

9. On the whole the different generations, including F_1 and F_2 of the various hybrid strains, showed a concordant tumor rate and tumor age.

10. If we consider our results as a whole, then, we may conclude that in crossing strains which differ in their tumor rate no rule of dominance applying equally to all cases seems to exist. In a certain number of crosses the results are undoubtedly intermediate. In these cases the tumor rate, and to some extent also the tumor age, behave in a manner similar to characters which differ in father and mother merely in quantity, as e.g., the length of organs. But from such intermediate results all kinds of gradations exist, leading on one side to dominance of the strain with the higher, and on the other side to dominance of the strain with the lower tumor rate. However, in our experiments,

dominance of the strains with the higher tumor rate happened greatly to predominate over the opposite extreme.

11. Our results on the whole are therefore in certain respects comparable to the inheritance of sex which R. Goldschmidt studied in hybrid strains of the gypsy moth. Here, also, all gradations from maleness to femaleness were observed in the offspring. Goldschmidt assumes that in different hybrids there are created different quantities of certain substances which, like enzymes, determine according to their quantity the velocity of chemical reactions and the amount of certain substances produced. These latter determine in the hybrids the quantitative variations in the character which is analyzed. According to Goldschmidt, multiple allelomorphs, which in our experiments seem to determine the heredity of spontaneous cancer, depend upon differences in the quantity of a substance present in the different individuals or varieties. In whatever way we may conceive of the character of multiple allelomorphs, our results make it very probable that multiple factors are involved in the heredity of cancer in mice.

DISCUSSION

Dr. Wells. I am glad that Dr. Loeb has brought up the question of inbreeding, because so many who are not familiar with this sort of work have the impression that inbreeding is the cause of the abundance of the tumors. Miss Slye's observations, however, agree with those of Dr. Loeb in showing that inbreeding itself is in no way responsible. When non-tumor strains are inbred, tumors do not appear because of the inbreeding. And when tumor strains are inbred, the incidence of tumors is, if anything, lower than it would be if they were cross bred, since inbreeding lowers the vitality of the stock.

Dr. Clowes: That observation corresponds with one made by Dr. Beebe in his experiments regarding the influence exerted on transplanted mouse tumors by eliminating carbohydrates from the diet. The animals which received carbohydrate-free diet were emaciated and the tumors grew far less rapidly than in the animals that had received a full normal diet. This appeared to be attributable to lowered vitality, malnutrition, or other conditions unfavorable for growth.

Mr. Marsh: We have in Buffalo a spontaneous mouse tumor strain which has reached the seventh generation. There has apparently been no change whatever in the high incidence of tumors through five generations. The sixth and seventh are below tumor age. This corroborates the experience of Dr. Loeb.

Dr. Loeb: I believe that the constancy of strains during continued propagation is of interest in another direction. Certain investigators, for example Haaland and Stahr, have described a change in the susceptibility of certain strains of mice for a tumor, after they had been

removed to another establishment and fed on a different diet. I have examined their evidence and I am not convinced that they were not dealing with a selection of certain families, rather than with an actual alteration in any one strain of mice. I do not believe that there is any evidence so far for the assumption that a change of diet or of climate can alter the character of a strain. Our own evidence shows that the various strains remain constant, and I believe that any assertion of a change should arouse a suspicion that selection of a certain family or of certain individuals has played a part in bringing about the variation.

10. A QUANTITATIVE STUDY OF THE PHYSIOLOGICAL ACTION OF RADIUM

Dr. Alfred Redfield (Boston):

SUMMARY

A physiological reaction well adapted to quantitative study is provided by the fact that the fertilization membrane of the egg of *Nereis limbata* becomes abnormally thick if the egg has been exposed to radiations from radium prior to fertilization.

The change produced in the egg by radiation is irreversible. The magnitude of the physiological effect is not proportional to the product of intensity and time of radiation, the time factor being relatively more important than the intensity factor. An equation expressing approximately the relation between intensity of radiation, I , and time, t , and their physiological effects, V , may be written: $V = a + b \log I + c \log t$. The constants, a , b , and c , depend on experimental conditions, but b is always less than c .

DISCUSSION

Dr. Wood: One interesting thing about these curves is their similarity to the curves obtained by plotting logarithmic density of photographic plates against times of exposure. There occurs what might be called the time of threshold exposure, which seems to exist in radium, in which you get practically no effect; and then the curve runs up, having a straight portion but ultimately falling off toward the abscissa as over exposure occurs. No doubt there is a certain physical similarity between the action of light and of radium, and I should like to ask if the radium curve also shows a falling off after high exposure.

Dr. Bowie: The general idea that the product of intensity and time is a constant in photochemistry is usually accepted more implicitly than the evidence warrants. There are a few reactions in gaseous systems, which are readily mixed, that follow the law; however, there are only four or five such photo-chemical reactions known. In the reactions of photo-chemistry the shape of the vessel is an important factor. This is a factor that in ordinary chemistry we do not have to contend with, except where the sides of the vessel have a catalytic action.

Dr. Clowes: I should like to ask Dr. Redfield what variation he observed in permeability with relation to the matter of conductivity. We found by determining the time factor on the permeability as measured by conductivity, in physical systems in which colloids are involved, that the curves were analogous to his curves. They were similar logarithmic curves, which ran parallel.

I should also like to know whether he observed any difference in the iso-electric point, if any, of the preparations.

Dr. Redfield: In answer to the question of Dr. Clowes regarding permeability, I would say that I have been unable to determine this point in these eggs. It seems almost obvious, however, that any change as great as the alteration I am measuring here must be accompanied by a parallel variation in the permeability of the membrane; but I have no evidence for that view. Nor have I any evidence concerning the iso-electric point in these eggs.

Dr. Clowes: Is it satisfactorily proved that the swelling of the membrane is a question of permeability? Is it a recognized fact that gelatin necessarily shows permeability?

Dr. Redfield: I cannot answer that question. Like Dr. Wood, I have been impressed by the similarity between this reaction and those of photography. The curves are identical with those illustrating the work of Hurter and Driffield on the effect of exposure of the photographic plate. They give a formula relating intensity and time of exposure to the resulting density of the negative, which is similar to the one which describes the reaction in *Nereis* eggs. In their formula there is a factor, i , which expresses the inertia of the plate; it is a measure of the difficulty of getting the process to start. This factor is represented by a function of the intensity of radiation in my formula. It is my idea that there is some secondary reaction which comes to a point of equilibrium determined by the intensity and which governs the rate of the primary reaction.

Another resemblance that is merely superficial is the fact that this is a process which is irreversible. It does not matter how long you wait with the egg before fertilizing it.

Dr. Wood: What was the result of very high exposures?

Dr. Redfield: The curve becomes very irregular.

Dr. Ewing: Were these effects due to the alpha, beta, or gamma rays?

Dr. Redfield: The radium emanations were contained in glass tubes which excluded the escape of the alpha, but not the beta and gamma rays.

Dr. Ewing: Were there any differences in the appearance of the cytoplasm of the egg?

Dr. Redfield: Not until the eggs were fertilized, when the abnormal membranes appeared. The resulting development was also abnormal.

Dr. Ewing: If Dr. Redfield will permit a practical man to make a suggestion, I would say that this paper strikes right at the central point of photo-therapy. I wonder whether he is familiar with work from the Middlesex Hospital on an identical problem, published in the Proceedings of the Royal Society. Not being a physicist, I was not able to follow the argument.

Dr. Redfield: I am not familiar with it. I notice that Dr. Prime and Dr. Wood present figures for the relative effects of intensity and time which follow this notion.

Dr. Wood: There is another factor, which is of importance in practice; that is, the phase of mitosis in which any cell happens to be. This gives another turn to the whole question of time and intensity. Enormous quantities of radium applied for very short periods of time are not nearly so effective as the same amount of radiation spread out over a long time; for in that longer time many more mitotic cells come under the influence of the rays, and it is the dividing cell that is most sensitive.

Dr. Redfield: It would introduce a variation in the same direction.

Dr. Wood: It suggests the use of long exposures wherever possible, as Professor Duane and I have both observed. The relative lack of success with the x-ray may be due to the short exposure, and the more pronounced effect of radium to a longer exposure. This question, however, has not been experimentally attacked, so far as I know.

Dr. William Duane (Boston): I should like to know whether this swelling of the membrane after the egg has been radiated is not a new phenomenon, quite different from anything else known before. If so, it should be carefully investigated.

Dr. Redfield: The reaction is not entirely new, having been very adequately described by Packard, in the Journal of Experimental Zoology for October, 1915. I have tried to duplicate it with other reagents. All of those which cause the membrane to swell do so whether the egg is fertilized or not. With radium the effect is produced only when radiation is followed or accompanied by fertilization.

I do not believe that there is anything very characteristic about the abnormalities obtained from radiated marine eggs. They resemble those produced by treatment with various reagents. Nor do I see any

great significance in the fact that eggs radiated during mitosis less often survive than those radiated between mitoses; for there is abundant evidence that the same kinds of egg are particularly susceptible during mitosis to many reagents (alkalies, chloroform, ether, etc.).

Fertilization is accompanied by an increase in permeability. After fertilization the permeability decreases again, to increase at the time of each cleavage.

11. ON THE MEASUREMENT OF x -RAY DOSAGE

Dr. William Duane:

SUMMARY

Several of the scientific commissions in this country that have been founded for the purpose of attacking the cancer problem from all sides are treating patients. At Harvard we see over six hundred patients a year, and at the Memorial Hospital in New York we see nearly one thousand a year. At least 95 per cent of these patients receive radiation treatments, either x -ray radiation, or radium, or both.

Further, a number of interesting researches have been announced on the effects produced by rays on living tissues. As this work progresses it becomes more and more important to estimate accurately both the quality and the quantity of the radiation employed. In view of these facts it does not seem out of place to present to this Association a brief outline of some of the investigations we have been making on the characteristics of the x -rays, and of the methods employed in estimating their penetration and intensity.

x -rays consist of trains of waves travelling out from the x -ray tube with the velocity of light c ($= 3 \times 10^{10} \frac{\text{cm.}}{\text{sec.}}$). The number of vibrations made per second is called the frequency, γ , and the distance from one wave to the next is called the wave-length, λ . Since, during one vibration, a wave travels along to the position occupied by the next preceding wave, it follows that during one second a wave will travel γ wave-lengths, or a total distance $\gamma \lambda$ cm. The distance travelled in one second, however, is the velocity. Hence we have the fundamental equation of wave motion

$$\lambda \gamma = c \quad (1)$$

The two most important characteristics of wave radiation are the wave-length (or frequency), and the intensity. Either the wave-length or the frequency may be used, as the other can be immediately calculated from equation (1).

By intensity of radiation we mean the amount of energy passing per second through a square centimeter of surface normal to the rays.

When electrons in the x -ray tube hit the target they produce the x -rays. The equation

$$V_e = \frac{1}{2} m v^2 \quad (2)$$

(V = voltage, m = mass of electron, e = its electric charge and v = its velocity) gives the energy of an electron. The impact against the target transforms only a small fraction of this energy into x -radiation.

In practical applications of x -rays a third characteristic becomes important, namely the co-efficient of absorption. This may be defined as the fraction of the radiation absorbed in the next thin layer of matter, taken either per unit thickness or per gram mass of the layer as convenience may dictate.

It is my purpose to state briefly certain simple relations between the above quantities that we have discovered recently.

An x -ray tube produces two kinds of radiation, the general radiation and the characteristic radiation.

Mr. Hunt and I found that the general radiation contains waves of all frequencies up to a certain maximum given by the energy relation

$$V_0 = h\gamma \quad (3)$$

where h is a well known physical constant appearing in the analysis of light. Thus the maximum frequency obtainable appears to be proportional to the voltage applied to the tube, and does not depend upon the characteristics of the x -ray tube.

The curves shown indicate the distribution of the energy of radiation among the various wave-lengths.

The frequencies of the characteristic radiation depend upon the substances composing the target in the x -ray tube, and not upon the voltage or current.

The curves shown illustrate the K series of lines of the chemical element rhodium. Particular interest attaches to the K lines of a chemical element, because they represent the highest frequency vibrations known to be characteristic of that element.

Dr. Webster, working in our laboratory, has shown that in order to produce the K series of lines the voltage must be at least as great as that given by equation (3) in which γ is the highest frequency of the series.

Professor Blake, Mr. Hunt, and I have measured the highest frequencies in the characteristic x -rays for over one-third of the chemical elements and find that approximately they obey the law

$$\gamma = \gamma_0 (N - 3\frac{1}{2})^2 \quad (4)$$

in which γ_0 is a constant taken from spectrum analysis, and N is the atomic number of the chemical element.

The atomic number of an element represents its position in the series of elements beginning with hydrogen as one and ending with uranium (the element of largest atomic weight) as 92.

The law connecting the velocity of the electron required to produce the K radiation with the atomic number appears to be exact within the limits of error of the experiments. It may be stated thus

$$N = N_0 (N - 1\frac{1}{2}) \quad (5)$$

in which N_0 represents a constant. The curves shown illustrate the laws.

Some years ago I collected all the data I could find bearing on the co-efficient of absorption of metals for x -rays, and worked out the relation between this co-efficient and the wave-length of the ray. The curve shown represents this data.

It appears that the co-efficient of absorption of an element increases as the cube of the wave-length, and may be expressed by the equation

$$N = k\lambda^3 \quad (6)$$

except near the characteristic lines of that element. For the mass co-efficient of absorption of aluminum $K = 14.7$.

Some time ago I designed an instrument for measuring the intensity and wave-lengths of x -rays. The drawings shown illustrate this instrument.

The x -rays pass through a small metal box and the ionization current through the air contained in this metal box is measured by means of a galvanometer. The reading of the instrument gives the intensity and the ratio of the two readings; a_1 with and a_2 without a thin sheet of aluminum (thickness d) gives the linear co-efficient of absorption from the formula $\mu = \frac{1}{d} \log \frac{a_2}{a_1}$. From this, by means of equation (6) the effective wave-length may be calculated.

By effective wave-length I mean a kind of average wave-length that has the same co-efficient of absorption as the whole x -ray beam.

DISCUSSION

Dr. Wood: I should like to have Dr. Duane tell us more about the calibration of the box.

Dr. Duane: It is a simple resistance box. With a given volume of air in it, one can calculate the intensity of the ray and the effective wave-length.

Dr. Redfield: I should like to ask Dr. Duane whether a similar method could not be developed for determining the quality of the tubes in the use of radium.

Dr. Duane: The only trouble is that the ionization current is very small, so small that one cannot measure it by the galvanometer. Other means are therefore necessary. The simplicity of this method consists in the use of the galvanometer, taking the reading on the scale. If one were to measure with some method involving the use of a stop watch, etc., the process would be more complicated, although the principle would be the same.

12. RECENT ADVANCES IN OUR KNOWLEDGE OF THE PHYSIOLOGICAL EFFECTS OF RADIATION

Dr. W. T. Bovie:

SUMMARY

Various theories have, from time to time, been advanced to explain the physiological action of radiation. These theories, since they are attempts to correlate observations with the aggregate of known facts, are but reflections of the philosophy of their times. The writer suggested in 1913, in discussing some experiments upon the temperature coefficient of the photo-coagulation of egg albumen, that the observed effects might be correlated by supposing that the radiation causes chemical changes in the more complex and unstable components of the protoplasm and that the subsequent physiological disturbances are the results of these changes. Undoubtedly, many students of the subject have entertained similar views. Nevertheless, in discussing the subject with investigators and clinicians it has often appeared to me that the ideas were not so clearly crystallized in their minds as in mine. The theory has been useful in directing my investigations and in interpreting my results, as well as the results of others. This paper is a brief statement of some of the results of a number of experiments calculated to throw light upon the nature of these photo-chemical changes and upon their relation to the resulting physiological disturbances.

It was pointed out in the experiments referred to above, that egg albumen becomes sensitized to heat when exposed to ultraviolet rays and coagulates at temperatures much below the "coagulation temperature" of the unexposed albumen. I have found that the protoplasm of *Paramecium* becomes sensitized to heat when exposed to ultraviolet (fluorite) rays and cytolizes at temperatures which are not above the optimum temperature for growth and reproduction of the unexposed organisms. The protoplasm of the fronds of *Laminaria Agardhii* is sensitized to heat by exposure to the rays from radium emanation, so that its resistance to the passage of an electrical current is very greatly decreased by brief exposures to temperatures which do not affect the non-radiated controls. It appears that heat sensitivity will be found to be a frequent peculiarity of radiated protoplasm.

I have found that it is possible, by selecting ultraviolet radiation of proper wave-lengths, to localize the place of action of these rays within the cell. The localization is due to the fact that extreme ultra-violet (fluorite) rays are largely, if not quite entirely, absorbed in the cytoplasm of paramecia, while the longer ultraviolet (quartz) rays are transmitted through the cytoplasm and are absorbed in the nucleus.

When a paramecium is exposed to fluorite rays (wave length in the neighborhood of 1600 Ångström units) for a sufficient length of time, it cytolizes. Sub-cytolytic exposures may be caused to give rise to cytolysis by a slight elevation of the temperature of the culture drop in which the organism is living (heat sensitization).

In one hour a paramecium so completely recovers from a brief exposure to fluorite rays that the effects of a second brief exposure are no longer reinforced by the remaining effects of the first.

Cell division is not inhibited by fluorite rays, however long the exposure. When a paramecium is exposed to quartz rays (wave length 2750 Ångström units) for a sufficient length of time it cytolizes. Sub-cytolytic exposures to quartz rays inhibit cell division. The duration of the inhibition increases with increase in the length of exposure. Short exposures cause brief periods of inhibition. Brief periods of inhibition are followed by acceleration, so that the number of descendants of the radiated paramecium may exceed that of the non-radiated control. With longer exposures, the inhibition may continue for several days. After long periods of inhibition the organisms do not recover, but become transparent and finally die.

The rays from radium also inhibit cell division in paramecia. In one experiment cell division was inhibited for seventeen days, after which division again occurred. In the meantime the control, dividing once every twenty-four hours, had potentially produced over one hundred and thirty-one thousand individuals. During this period of inhibition the radiated organism became thin and transparent and lost its power of locomotion.

The results of this experiment are interpreted as indicating that the physiological effects of the radiation are the results of photo-chemical changes taking place in the protoplasm at the place where the rays are absorbed.

DISCUSSION

Dr. Clowes: I am interested in Dr. Bovie's curves with sodium and calcium because I am going to exhibit somewhat similar curves obtained with purely physical systems containing no proteins, but consisting simply of oil, water, soap, and filter paper, in which, consequently, the effects observed cannot be attributable to any action on proteins.

Dr. Bovie's results suggest colloidal effects. For example, it is well known that the aggregation of a colloidal suspension may be effected far more readily by adding a given amount of precipitant at one time than by adding the same amount divided in several instalments. The irregularity in curves followed by subsequent reversion to the normal observed by Dr. Bovie is something with which we are already familiar in experiments both upon living cells and physical systems under the influence of electrolytes. The close resemblance between the proportions of certain electrolytes in sea-water, in the blood of mammals, and in other envioning media of protoplasm, suggests that protoplasm may represent an adaptation of certain colloidal constituents to their original envioning medium. The difficulty of getting results with physical systems which perfectly parallel those obtained with living cells may be attributable in a measure to the extraordinarily fine

balance attained by protoplasm during the long period of time occupied in the process of evolution. An emulsion prepared ten years ago exhibits superior properties to one recently prepared, and when it is contained within the meshes of filter paper, jelly, or other carrying medium, the curves exhibited are much more perfect than when a crude emulsion is employed.

Dr. Bovie: As for the physical changes involved in cytoplasm I may say that while studying the photo-cytolysis of paramecia by fluorite rays I endeavored to find a physical explanation of the phenomenon. The visible changes had such a strong resemblance to the breaking down of emulsions that I decided to investigate the effects of rays upon emulsions. I discussed the matter with physicists, and was assured that the experiments would give negative results because it was impossible for radiation to affect surface tension sufficiently to affect the equilibrium of an emulsion. I had, however, observed that small drops of water, paraffin oil, carbon tetrachloride, or in fact, of any of the substances that I tried, could be made to creep about by radiating them with fluorite rays. I made up emulsions of olive oil and dilute alkali and found that they broke down very quickly when radiated with fluorite rays. It was possible actually to bore a hole through a stiff "mayonnaise-like" emulsion made by beating olive oil and dilute alkali. I have not found an adequate explanation of the breaking down of these emulsions, and have never published the experiments. I am not supposed to be able to explain the mechanism of the photo-cytolysis of paramecia, but it does not redound to my credit that I am unable to explain so simple a thing as the breaking down of an emulsion of soap and water, by fluorite rays.

Dr. Wood: I feel that we are all rewarded by this discussion, which has been very interesting to me as evidence that we are coming closer to some possible explanation of what is going on inside the cell. Dr. Bovie's work on the paramecium and its nucleus interests me particularly because I have long held the belief that too small doses of radium stimulate the tumor cell, while larger ones may check its growth. Tumors, however, are such fluctuating material that it is difficult to advance an absolute proof.

Dr. Bovie: I am unable to offer an explanation of the stimulating effects of the rays. I have tried to obtain a stimulation without an inhibition but so far have not succeeded. A stimulation following an inhibition strongly suggests the blocking of a series of reactions, the stimulation occurring when the blocking breaks away.

13. RECENT CHANGES IN THE MORTALITY FROM CANCER

Dr. Frederick L. Hoffman (Newark):

SUMMARY

In my treatise on "The Mortality from Cancer Throughout the World," the final conclusion was advanced, and without hesitation, that "the evidence of cancer increase throughout the world is an incontrovertible statistical fact and absolutely conclusive." During the intervening three or four years a considerable amount of new information has been forthcoming but no evidence has been produced to necessitate a modification of the conclusion that the observed increase in the cancer death rate throughout the world is real and not apparent or in consequence of improved diagnoses, changes in the methods of death certification, etc.

The human death rate is such a composite factor of human existence that it would be hopeless to contend for universal laws or principles governing specific disease occurrence proportionate to the populations affected, with a due regard to age, sex, and racial distribution. Extended statistical analysis never fails to bring to light new contradictions in cancer mortality experience but in the main the conclusion would seem entirely sound, and in strict conformity to the facts, that "cancer is much more common than has generally been assumed to be the case," and that "the mortality from the disease throughout the civilized world exceeds 500,000 deaths per annum, and is in the United States about 80,000 at the present time." Efforts have been made from time to time to contradict the conclusion that the increase in the mortality from cancer is real, and not apparent, but the evidence brought forward can not be considered satisfactory for the purpose, more so in view of the fact that the proof has been statistical rather than medical or surgical, indirect rather than direct, or general rather than specific.

In a recent contribution to The Journal of Cancer Research, Prof. W. F. Willcox, of Cornell University, has revived the controversy by a presentation of new data for the city of Frankfort, in continuation of the earlier analysis by Newsholme and King. The argument is entirely statistical and, broadly speaking, without real medical or scientific value in the vast field of cancer research. The data as such, which are quite extensive in numbers and point of time, were not subjected to critical medical consideration by one qualified to do so, and there is nothing to indicate that each and every death certificate was gone over with the necessary care. In fact, very little is made of the new Frankfort data by Professor Willcox, and most of his discussion has reference rather to general questions of mortality analysis—that is, variations in the death rate on account of variable factors such as age and sex distribution, etc. The misleading classification adopted by Newsholme and King is adhered to regardless of the obvious urgency

of a more precise and scientific differentiation of cancer recording as to accessibility or inaccessibility, for purposes of initial or terminal diagnosis. The cause of cancer research is hindered rather than advanced by mere controversial discussions of this kind, which, at least to the general student of the subject, must make confusion worse confounded.

It is not my intention to review the article by Professor Willcox in all its essential details at the present time, nor would such a discussion serve a very practical purpose where so much really useful work requires to be done. The article bears intrinsic evidence of an essay in statistical controversy rather than an attempt to further important purposes in connection with cancer research. Since one of the main conclusions implied is that the apparent increase in cancer is probably chiefly in consequence of improved diagnosis, it need only be said that no specific evidence whatever is advanced that cancer diagnosis has been seriously at fault in any special branch of cancer mortality statistics; for, after all, real progress in cancer mortality analysis can only lie in the direction of a thoroughly critical and qualified consideration of carefully selected groups of cancer facts, such as cancer of the breast, the buccal cavity, the uterus, etc.

Professor Willcox prefers the dual classification of accessible and inaccessible cancers adopted by Newsholme and King on the basis of an obviously faulty classification, to the strictly scientific method suggested by Dr. Bashford, of the Imperial Cancer Research Fund, including an intermediate group of borderline cases clearly belonging to neither one nor the other. Although preferring the dual classification to the triple classification, Professor Willcox, in his own data, includes a group of cases with the position undefined, which is sufficiently large to invalidate practically all of his conclusions, at least for the earlier years. For illustration, during the years 1860-1866, out of 1603 cancer deaths in the city of Frankfort, 359, or 22.4 per cent, represented deaths in which the position was undefined. Although "for the sake of simplicity" Professor Willcox gave the preference to the dual classification, he certainly was not justified in referring to the deaths assigned to the position "undefined" as "*insignificant*." Furthermore, his admission that arbitrary assignments in the dual classification as followed were unavoidable overlooks the fact that certain cancers, such as those of the skin, other parts of the mouth than the tongue, and other external parts of the body, which should have been classed as accessible, were not so classified because they were not distinguished in the printed sources as used by Newsholme and King, is certainly not a scientific argument. To draw false inferences because of inherently faulty data does not advance the cause of scientific research.

The use of the Frankfort data in connection with cancer mortality investigations is one of the curiosities in medicine. Why so much pains should have been taken with an analysis of statistics practically inaccessible for verification, and made in a city subject to entirely different methods of registration practice, death certification, etc.,

rather than any one of the larger cities of this country, is inexplicable. The tabulation of the original data as, fortunately, included in an appendix to the article by Professor Willcox, clearly shows that the information is thoroughly self-contradictory throughout the whole long periods for which the data have been brought together. No such classification as is followed by the city of Frankfort is in current use in this country or in any other country which has adopted the international classification. The number of cancers with the position undefined is 688, out of 9242, but there are other cancers specifically defined, such as those of the nervous system, the bones, etc., which do not admit of a differentiation as to whether accessible or inaccessible, as the case may be. If, therefore, any real value is to be attached to the Frankfort data they should be entirely rearranged and a complete tabular presentation should be made according to the organ or part of the body affected. As thoroughly made clear in my discussion of methods of classification, etc., consistent uniformity is of the first importance.

Qualified observations on the increase in cancer are practically co-extensive with the progress of medicine, at least during the last one hundred and fifty years. It would be truly inconceivable that, say four or five generations ago, cancers of all forms, organs, and parts should have been as common as at the present time without the fact having sufficiently attracted the attention of the medical practitioners of the period. Superficial conclusions regarding erroneous cancer diagnosis almost invariably overlook the fact that the diagnosis is much less difficult *at death* than is generally assumed to be the case, however difficult the initial diagnosis of cancer may be in the case of any particular organ or part. This conclusion applies in a large measure to internal cancers as well as those of the external, or readily accessible internal, organs or parts. So careful an observer as Christopher Turner Johnson, in a practical essay on cancer to which was awarded the annual prize for 1808, by the Royal College of Surgeons, and of which an American edition was printed at Philadelphia in 1811, described only cancer of the breast, the uterus, the generative organs, the skin, the eye, and the tongue; yet even Johnson, who dedicated his essay to Astley Cooper, the most eminent surgeon of his time, gives expression to the opinion, based on an examination of the Bills of Mortality, that "the proportional number of deaths from cancer in a given time has of late years been on the increase." This increase has been so very considerable and so persistent from year to year that it would be truly inconceivable that material medical or statistical errors should invalidate the conclusion that cancer at the present time, in proportion to population, is relatively twice as common as it was forty years ago.

Arguments that cancer is not on the increase may conversely be construed as conclusive proof that cancer is on the decline, for no death rate from any cause ever remains stationary, but continuously fluctuates, with generally either a pronounced upward or downward

tendency. The cancer death rate, even for recent years, has not always shown an increase, and it is extremely significant that during the year 1917, for illustration, the cancer death rate of 35 American cities should for the first time in eleven years show a distinct downward tendency. As shown by the table following, the death rate has almost continuously increased from 77.8 in 1907 to 93.9 in 1916, per 100,000 of population, but it declined to 92.8 per 100,000 during the year 1917, regardless of an enormously enhanced public interest in the cancer question, both on the part of the medical profession and the laity. If, therefore, the argument so frequently advanced, that the increase in cancer is in consequence of improved diagnosis, then it is a fair assumption that the same authorities would hold that the mortality rate from cancer had decreased, for the opposite reasons.

Mortality from cancer in thirty-five American cities, 1907-1917

YEARS	POPULATION	DEATHS	RATE PER 100,000 OF POPULATION
1907	15,491,267	12,045	77.8
1908	16,014,235	12,302	77.4
1909	16,464,980	13,413	81.5
1910	17,012,722	14,206	83.5
1911	17,442,980	14,562	83.5
1907-11	82,426,184	66,618	80.8
1912	17,895,192	15,367	85.9
1913	18,322,037	16,393	89.5
1914	18,756,577	16,955	90.4
1915	19,209,832	17,306	90.1
1916	19,636,951	18,439	93.9
1912-16	93,820,589	84,460	90.0
1917	20,113,139	18,657	92.8

The details of this analysis, by single cities, are not available at the present time further than that, comparing the 1917 rate with the average rate for 1912-16, the rate increased in 24 out of the 35 cities, the remaining 11 cities in which the rate decreased having been Buffalo, Dayton, Hartford, Indianapolis, Milwaukee, Newark (N. J.), New Orleans, Paterson (N. J.), Richmond, Seattle, and Worcester. The favorable changes in the death rate during 1917, however, are much more pronounced when the record for the year is compared with the corresponding rates for 1916. It therefore admits of no controversy that exceptions to the general principle of cancer increase may be met with for short periods of time, although the general trend of the rate for the large majority of representative localities may continue in an upward direction.

It admits of no argument, of course, that the death rate from any specific cause must at some time reach an approximate maximum point

of relative frequency. Considering the fact that a perceptible number of deaths from cancer are annually prevented by surgical operations, or otherwise, and in all probability to a much larger extent at the present time than in former years, the observed increase in the cancer death rate is so much more significant. Considering further that in certain countries like Switzerland the cancer death rate exceeds 100 per 100,000 of population, or 1 per cent, whereas in this country, for the registration area, the rate has not yet reached 80 per 100,000, and for the 35 cities only 90 per 100,000 during the five years ending with 1916, it is self-evident that we are approaching a point of maximum frequency for the country at large which may be conservatively placed at 120 per 100,000 of population, although rates have been reported in excess of that amount for a number of thoroughly representative countries and localities. The European war will necessarily impair all future cancer statistics, and a correction for profound alterations in the age and sex constitution of the populations concerned will become most difficult, if not statistically impossible. Even in the case of our own country, allowance for population changes will have to be made in the future in consequence of our participation in the European war, although, of course, to a much lesser extent than, for illustration, in the case of Germany, Austria, Belgium, and France.

Without further enlarging upon the question, I can not but feel that the evidence of a material increase in the cancer death rate during the last generation is sustained by such an overwhelming mass of statistical evidence that statistical arguments to the contrary belong to the realm of hopeless conjecture and idle speculation.

DISCUSSION

Dr. James Ewing: I think it is remarkable that two such distinguished and unquestionably accomplished statisticians as Dr. Hoffman and Dr. Willcox, dealing with a clean-cut question and having at their disposal much the same data, should come to conclusions which are not in unison, and which, in fact, are in some respects diametrically opposed. There must be some clear and definite reason for this difference. I do not pretend to know just what it is, but I have an idea that Dr. Hoffman hit the nail on the head in his last sentence, when he attributed it to the incompetent work of the physicians on whom a part of these statistics depend. I do not want to destroy Dr. Hoffman's confidence in the diagnostic ability of his physician, or of physicians in general; I do not want to underestimate the capacity of the medical profession to diagnose; but I am quite sure that if Dr. Hoffman were as familiar as we pathologists are with their inaccuracy, even under the best circumstances, I do not think he would be quite so severe on Dr. Willcox's conclusions. We are only too well acquainted with the difficulty of getting reliable information from any considerable proportion of men even of average medical intelligence. But I do not think that Dr. Hoffman understands the great complexity of the

disease that we include under the term cancer, or that he is familiar with the medical side of the problem. While I am in no sense an apologist for Dr. Willcox I strongly advise all who have not read his article to do so. I was more or less responsible for the general color of Dr. Willcox's views, and spent a considerable amount of time in explaining to him the sources of error in his statistical data. I think it possible to develop that subject even more than he has done, yet just as the matter stands now he assumes that over one-half of the recorded increase can be explained on this basis. In fact, Dr. Willcox goes so far as to say that the remainder of the increase may possibly be susceptible of similar explanation.

As a matter of fact, the subject of the increase of cancer is, in my opinion, not ripe for purely statistical study. In order to reach proper conclusions, it is necessary to study the psychology of the doctor, the psychology of the public, and a great many other complex factors. This is necessary, in order to determine whether a patient actually did die of cancer when the death is recorded as such.

Finally, as to the deplorable expense mentioned by Dr. Hoffman, we spent only one hundred and fifty dollars in this investigation, for which I was glad to be able to secure the funds.

Dr. R. A. Lambert (New York): Dr. Ewing has expressed my own ideas much better than I could have presented them. It seemed to me, as Dr. Hoffman was speaking of the crudity of the data, that if any sum of money was to be expended it should go toward improving the data, and not toward investigating the crude data that we now have.

There are limitless possibilities for improvement of the statistics. Thus data are to be obtained that are not open to the sources of error emphasized by Dr. Ewing. These are autopsy data, which are probably included in the material that Dr. Hoffman has analyzed; I am not sure that they are kept separate. The New York Board of Health follows the excellent plan of sending blanks to the hospitals, which the pathologist fills out after having completed his histological examination. I do not know whether the data thus obtained are kept separate from the other statistics, in which the source of error is great, but it should be. It seems to me that a city like New York could accumulate in ten or fifteen years statistics that would be extremely valuable, and the plan might be followed in other cities to great advantage. Again, a law might be passed providing for an autopsy in the case of every person who dies of anything that might be cancer, though I suppose this is too much to hope for in this country.

Dr. Woglom: I have no preconceived notions on the question whether the number of deaths from cancer is increasing or not, and ask the following question merely for my own information. It has been suggested that if the rising curve representing a constant increase in the cancer death rate were to be produced backward, it would, sooner or

later, reach zero; in other words, at some time in the past there may have been no deaths from cancer. I wish to know whether a statistician would regard this as a reasonable criticism of the view that the number of deaths from cancer is increasing.

Dr. Wood: An interesting example of the reaction between the physician and the diagnosis is now being observed in England. The number of deaths ascribed to cancer is increased over the records of the most recent preceding years, but the deaths from sarcoma are fewer. In the report of the Registrar General it is pointed out that the decline in sarcoma mortality is probably associated with the increase in that from undefined cancer, for the former had been increasing and the latter decreasing for many years up to the commencement of the war, since which time the movement seems to have been reversed. The most probable explanation, according to the Registrar General, may be found in the withdrawal from civil practice of so many of the younger physicians, who would probably distinguish sarcoma as such in their certificates somewhat more frequently than the older men, upon whom the added burden of their work has now fallen. If we did not know what is going on in England we should be at a loss to understand the reversal of the figures; and this is a concrete illustration of how our statistics vary under different methods of investigation.

Dr. Hoffman: In reply to the statement by Dr. Ewing that perhaps the question of an increased cancer death rate cannot (if I quote him correctly) be properly dealt with statistically, I would say that I might be inclined to agree with him if the analysis is to be made by men who have no medical knowledge and understand only statistics. Nothing is more misleading than mere statistics, and most of the analogies fail precisely on that point.

A very interesting question was raised by one of the speakers, who inquired as to the justice of producing the curve backward. I think that this would be a fair procedure, and that the fact that it would reach zero indicates that cancer is a disease of modern life. There are many competent observers who insist that cancer is rare among primitive people, and who are therefore certain that cancer is a modern disease.

Again there is reason to believe that the great range of frequency as regards the different parts of the body affected, gastric cancer being especially common, is a modern phenomenon due to completely new modes of life. Surely, if those who have practiced for a generation among Indian tribes have not been able to find any evidence of the occurrence of cancer in them, or to discover any evidence of death from malignant disease of the bones by examining their remains, it is a reasonable assumption that the disease must be rarer in this race than in others. Now I am by no means opposed to the idea that in primitive people of past generations cancer has occurred; but it must have been very rarely.

When we examine the records of the past, we find that the increase of cancer has long been a subject of discussion. Thus, in a book written by a prominent English physician one hundred years ago, and dedicated to Sir Astley Cooper, himself the author of a book on cancer of the breast, the following sentence occurs: "It appears that the number of deaths from cancer in a given time has, of late, been on the increase." In the older records of Vienna, cancer was only mentioned once in four or five thousand deaths; and the literature of two hundred and fifty years ago shows that while cancer was recognized, it was referred to much more rarely than at the present time.

I would not oppose the idea that there may have been a time when cancer was actually at the zero point. Its modern increase I believe to be due, as I have already said, to the extremely complex conditions of modern life. The responsible factor may be an error in metabolism, an excess or deficiency of some mineral salts in the diet, or the ingestion of some irritating substance; or there may be a multitude of causes. There is no reason at all, in my opinion, to oppose the idea that cancer has increased; indeed the wonder is that it has not increased much more, when we contrast modern habits of eating with those that prevailed even earlier in my own lifetime, forty or fifty years ago.

I am well aware that this is by no means an answer to the question of the increase of cancer; but it is an indication that these factors have not been overlooked by those who are committed to the view that cancer is actually on the increase.

Something was said about autopsies. It may interest the Association to know that all the autopsy records of the Johns Hopkins Hospital are in my office and have passed through my hands. They are extraordinarily complete as to detail, and I am sure that no German or Austrian hospital can equal them in this respect. All have the microscopic findings attached. In other words, so far as any reasonable statement of facts can go, I believe that the Johns Hopkins data are practically conclusive; and while their analysis has not yet been completed, it has gone far enough to discount the alarming statements of those who would indict the whole medical profession after discovering a few cases of undiagnosed cancer.

I realize fully that one who is not an experienced physician; and, more than that, an experienced surgeon; and still more than that, an experienced pathologist, microscopist, and biologist, as one should be in addition to his qualifications to deal with statistics, is greatly handicapped; and I have therefore been reluctant to commit myself too deeply, knowing my lack of medical knowledge. But there is not a leading surgeon from Maine to California with whom I have not discussed this question; I have seen one hundred operations for cancer in the Mayo Clinic alone; I have examined the records of the University of Minnesota, and I am satisfied that we shall never get anywhere by a mere statistical analysis and re-analysis of foreign data. The only hope of progressing anywhere lies in a thorough examination of the facts under our own control, within our own experience, in our own country. Even though we were limited to a few thousand cases, we should then advance, instead of being led into useless controversy.

14. (a) PRELIMINARY NOTE ON THE ACTION EXERTED BY ANTAGONISTIC
ELECTROLYTES ON THE ELECTRICAL RESISTANCE OF
EMULSION MEMBRANES

Dr. G. H. A. Clowes:

SUMMARY

Previous determinations have shown a high content of K, Na, and soap, and a low content of Ca, in rapidly growing tumors; and since the permeability of normal tissues is known to be increased by salts of Na and K, and decreased by those of Ca, it appeared desirable to determine the influence exerted by the electrolytes in question on the conductivity of purely physical emulsion systems of the type previously described by the writer. When solutions of NaCl and CaCl₂ having an equal conductivity were allowed to act on emulsions contained between filter paper diaphragms, NaCl increased and CaCl₂ diminished the conductivity; and it was found possible by means of alternating treatments with the salts in question to produce rhythmical variations in conductivity corresponding exactly with those observed by Osterhout in his experiments with marine organisms.

That the electrical conductivity affords an accurate index of the permeability was proved by passing the solutions in question through emulsion systems, when it was found that NaCl greatly increased the speed of flow and CaCl₂ gave a result approximately the same as that given by distilled water or sea water.

These results, together with those recently published by Hirschfelder, correspond with the writer's experiments on antagonistic electrolytes carried out by means of the drop system, and support the contention previously advanced that variations in permeability of the protoplasmic membranes are attributable to the action of electrolytes and metabolic products on a delicately balanced emulsion system, and that proteins simply afford a supporting structure for the mechanism in question. This view is supported by the fact that blood plasma exhibits only a slight variation in conductivity, either on clotting or on subsequent treatment with antagonistic electrolytes.

The fact that emulsions, when admixed with CaCl₂ without a filter paper diaphragm, give only variations in conductivity as compared with those carried out with a diaphragm, indicates that the contact mechanism lies in the emulsion in the pores of the filter paper diaphragm.

14 (b) PRELIMINARY NOTE ON ELECTRICAL RESISTANCE OF TUMOR
TISSUES

Dr. Clowes:

SUMMARY

Preliminary determinations of electrical resistance indicate that cancerous tissues are more permeable than normal tissues, and that the permeability appears to be directly proportional to the speed of

growth. Similar determinations on plant galls supplied by Dr. Erwin F. Smith, indicate that plant tumors are more permeable than normal plant tissues. This method might afford a means of determining the relative malignancy of tumors.

DISCUSSION

Dr. Bowie: I should like to call Dr. Clowes' attention to the fact that Dr. Brooks has recently published some experiments in which he used sections of laminaria instead of filter paper, and obtained results similar to those reported by Dr. Clowes. Sodium chloride passes through very quickly, while balanced salt solutions pass through much more slowly or not at all.

Dr. Redfield: The curve shown by Dr. Clowes, which represents first a decrease and then an increase in permeability, is approximately the form of the differential of the equation expressing the integral of the effect of radium on the egg of *Nereis*; and this fact seems to suggest some fundamental underlying principle in both cases.

There is a question I should like to ask Dr. Clowes concerning the work of Zwaardemaker, which has recently appeared in the American Journal of Physiology. He found that he could replace the potassium in Ringer's solution by a number of radio-active substances, provided the quantity was sufficient to be just as radio-active as the potassium omitted, and still maintain a balanced physiological solution. He concluded from this that the physiological action of potassium was due to its radio-activity. I wonder whether that was a justifiable conclusion, or whether it would be better to think of potassium and of radiations as both increasing permeability, and so being antagonistic to other ions which decrease permeability.

Dr. Duane: I should like to ask Dr. Clowes whether or not he has used iridium salts. They are much more radio-active than potassium.

Dr. Clowes: The conductivity results with the filter paper systems have, in our experience, proved more reliable than those obtained with living tissues. The results on conductivity with plant tissues are far more satisfactory than with animal tissues; nevertheless, the method may be suggested as one which might be utilized by pathologists to determine the relative speed of growth and the malignancy of a given tumor. The fact that embryonic tissues exhibit greatly increased conductivity, and that marine organisms subjected to toxic influences, unfavorable chemical environment, sewage, etc., likewise exhibit an increased conductivity, suggests the possibility that investigations on these lines might throw some light on the cancer problem.

Without introducing any further mathematical discussion, it may be stated that the physical systems described above, reacting as they do

with calcium, magnesium, sodium, potassium, etc., appear to parallel living tissues closely and should afford some information regarding protoplasmic structure.

I have not used iridium on the systems just described. There is much in radio-activity that suggests the advisability of investigating the effects produced in their relation to the action of adsorbed hydroxyl ions in colloidal systems.

As regards the antagonistic effects exerted by sodium and potassium, on the one hand, and calcium and magnesium on the other, it should be noted that, in surface tension experiments with capillary pipettes and soap films, under approximately neutral conditions sodium and potassium antagonize calcium and magnesium; with increasing alkalinity magnesium appears to function, like sodium and potassium, as an antagonist to calcium; and with increasing acidity potassium tends to antagonize sodium. It is therefore very important to consider the adsorption of hydrogen ions and hydroxyl ions in such colloidal systems.

Dr. Bovie's experiments with potassium remind me of certain experiments which we made some time ago with soap jellies containing varying proportions of sodium and potassium. When such jellies contain only sodium they are solid at the normal temperature of sea-water, but the addition of a small amount of potassium equal to that occurring in marine systems produces a semi-fluid jelly at the temperature in question. The extent to which the melting point of such a jelly may be raised by the addition of a small amount of potassium is really remarkable.

The influence of temperature on Dr. Bovie's curves would certainly appear to indicate that he is not dealing with an ordinary chemical reaction in a homogeneous system, but with a colloidal system in which adsorption and surface tension effects come into play. While chemical reactions exhibit an increase in velocity with rising temperature, surface tension diminishes in a manner corresponding with Dr. Bovie's curves.

Dr. Ewing: These physicists remind me of the advice that, "Even in philosophy, it is necessary to use terms with meaning." I have no doubt that the terms are full of meaning to them, and that they refer to definite facts in physics; but I have listened to similar discussions in this society for several years, yet have listened in vain for any attempt to translate these terms for the pathologist. Is it not possible for the physicist to explain pathological changes from the view point of the physician, and to interpret phenomena from this standpoint, thus transforming the discussion from one largely meaningless to the pathologist into one that is full of significance? If this can be done, you may be sure of introducing some important changes into pathological interpretation, for the physicist can explain many of these things better than the pathologist can.

Dr. Bovie: I want to ask Professor Ewing whether the material that I have attempted to present this afternoon was unintelligible; for I really tried to eliminate all the terms that I thought would not be understood. Handicapped as I was this morning by pathological terms, I can fully realize the difficulties that confront one who is listening to a paper outside one's own field, even when the speaker strives to employ only such terms as belong to the ordinary scientific category. However, I challenge Dr. Ewing to prove that it was the mathematics that was not intelligible.

Dr. Ewing: Not from the physical standpoint; but the point that I was trying to make is that one can neither see these things going on in the living cell nor interpret the morphology in the terms that have been employed here.

Dr. Clowes: We have tried to investigate questions of surface tension in the amoeba, for example, and also to interpret, in terms of actual physical equilibrium of a two or three phase system, the phenomena of karyokinesis. But until one has one's self some sort of definite idea of what one is driving at, it is not easy to employ terms that shall be intelligible to another.

I think that what I did not make clear to the pathologists is this: A particle that is suspended in water—oil, say—may be dispersed by an agent that lowers the surface tension. When we use soap, we disintegrate the particles of oil and spread them out. A little salt will further disperse the oil and lower the surface tension, and give more and more of these particles; because the negative chlorine ion is more active than the positive sodium ion.

I am going to send Professor Ewing some articles that I have written, and ask him whether they are unintelligible. If so, I shall start all over again. I want to put before pathologists the material that we have worked on, for I realize that I myself cannot apply it, and that I must leave the application to men like Dr. Ewing.

15. THE RELATION BETWEEN SPIROCHETES AND MOUSE TUMORS

Dr. Theodor Mueller (Buffalo):

In 1905 Borrel found spirochetes in nonulcerated mouse tumors, but apparently did not attribute much significance to them. Wenyon, and Breindl and Kinghorn, in 1906, found spirochetes in the blood of non-tumor mice which had been experimentally infected with trypanosomes, and had no difficulty in transmitting the spirochetes subcutaneously and intraperitoneally to other mice. In 1907, both Gaylord and Calkins described spirochetes in mouse tumors. Gaylord saw them in sixteen secondary tumors (adenocarcinoma) from three different strains. They were most numerous at the margin and in the connective tissue of the tumors, only a few being discoverable in the

center. These authors succeeded in finding spirochetes in ten primary tumors also, though never in any tissue of healthy mice, and therefore suggested that these organisms probably bore some etiological relation to the genesis of mouse carcinoma. Soon afterward Tyzzer described spirochetes in four mice, one of which had been successfully inoculated with tumor, while the other three were immune. Deetjen, in 1908, saw spirochetes in two different strains of tumor mice, and also in the subcutaneous tissues of mice which were immune against tumor inoculation. But he could not find spirochetes in healthy mice or in mice with primary tumors; and as he observed the development of a secondary tumor without spirochetes, he reached the conclusion that in all probability the spirochetes had nothing to do with the etiology of tumors.

The present experiments were undertaken with the object of definitely clearing up the relation between spirochetes and mouse carcinoma. In these experiments, only mice from the laboratory of the State Institute for the Study of Malignant Disease were employed. All transplantations of tumor were made with a trocar, which was introduced into the subcutaneous tissue of the back. The material to be inoculated was derived from an adenocarcinoma (R. I. 33) which shows numerous spirochetes in sections impregnated by the Levaditi method. In the search for spirochetes the dark field was usually employed, though Giemsa's and Fontana's stains, Loeffler's flagella stain, and India ink preparations were also tried. Peritoneal fluid, blood, smears and fresh teased preparations of normal and carcinomatous tissue, and sections impregnated by the Levaditi method were examined.

The spirochetes prevalent in our mice are most numerous in the peritoneal fluid, where hundreds could sometimes be seen. They were also present in the blood, and occasionally in the pleural cavity. Their average length is 4 to 6 microns, though some measure only 2.7 and others as long as 7.3 microns; both of these extremes however are met but rarely. The number of gyrations is usually 4-5; some have only 3, and the longest may have as many as 9 gyrations. The spirochetes show a very marked motility, which seems greater in the blood than in the peritoneal fluid. They all have a broad flagellum on each end which is plainly visible by dark field illumination and sometimes seems closely connected with a periblast-like structure. The spirochetes can be demonstrated in smears without difficulty with aniline dyes; the flagellae can be stained by Loeffler's flagella method, but do not appear to be demonstrable with the Giemsa stain. India ink preparations show the flagellae plainly, but they are not stained in sections impregnated by the Levaditi method. The spirochetes in these sections resemble closely those described by Gaylord and Calkins and, I have no doubt, are identical with them. These authors were unable to stain the spirochetes by the Giemsa method but their lack of success is easily accounted for by the fact that they tried to stain the organisms in sections. Those now being described cannot be stained in

sections by the Giemsa method either, though they take this stain readily in smears.

The spirochetes can be transmitted to other mice subcutaneously and intraperitoneally without difficulty. This makes it probable that Deetjen saw the same spirochetes; and the probability is supported further by the fact that Deetjen's spirochetes were about the same size, had flagellae, and were prevalent in immune and non-immune laboratory mice. The description given by Wenyon and Borrel does not exclude the possibility that they also were dealing with the same organism. Whether this is closely related to the *Spirocheta morsus muris* (Futaki), which is pathogenic for men and with which it corresponds in several respects (staining properties and flagellae) can not yet be decided. The comparative results of cultural experiments and of agglutination and immunization tests must be awaited.

Numerous spirochetes were found in all mice inoculated with tumor, whether they were immune or not. To discover the source of the spirochetal infection, 46 healthy spirochete-free mice were inoculated with tumor, twenty spirochete-free mice being used as controls. Forty-three of those inoculated (93.5 per cent) showed spirochetes in the peritoneal fluid and in the blood after four to ten days. There was no distinction between successfully and unsuccessfully inoculated mice. All the developing tumors contained spirochetes. Of the control animals, only two showed single spirochetes in the peritoneal fluid after nine days. The result of this experiment points strongly to tumor inoculation as the cause of the infection.

However, another possibility had to be considered; namely, that the spirochetes might live in the superficial skin lesions caused by those parasites of the skin which are so prevalent among mice. With the instrument used for inoculation they might then be deposited in the subcutaneous tissues and so cause a spirochetosis. A sterile trocar was therefore introduced empty into the subcutaneous tissue of 18 spirochete-free mice and immediately withdrawn. But in none of these mice could spirochetes be detected, even after weeks.

As mentioned above two control mice showed spirochetes after nine days' observation, and one of the numerous bugs (*Acanthia lectularia*) infesting the cages therefore came under suspicion as the transmitting agent. A number of these insects were accordingly caught and fed with blood containing spirochetes, and after various intervals (five days to four weeks) allowed to feed on healthy mice. No spirochetes could be found during an observation period of four weeks, but all the mice of this experiment were then unfortunately lost, except one. This remaining one showed spirochetes two months after exposure to the bugs.

These experiments prove that it is inoculation with tumor R. I. 33 which is the almost exclusive cause of the presence of spirochetes in our tumor-bearing mice.

What, then, is the relation between these spirochetes and the tumor? Both Gaylord and Calkins suspected them to be the causative agent,

and point to their regular prevalence in transplanted and sometimes in primary tumors, and to the fact that they are always most numerous at the margin of the tumor, where probably the most intensive cell-proliferation is in progress. On the other hand, it is not without significance that the same spirochete is as common in immune mice, in which transplants do not develop; and it is further to be considered that spirochetes can be transmitted to other mice subcutaneously and peritoneally without difficulty. It was therefore important to investigate the prevalence of spirochetosis among non-inoculated mice. Deetjen, who examined 25 healthy mice, found no spirochetes, and Gaylord and Calkins examined a small number of normal mice, also with negative results. Only Breindl and Kinghorn report that they found two mice infected with spirochetes among six wild mice. I have examined 324 normal mice of our laboratory stock. In six of them (1.85 per cent) spirochetes could be found in the blood, in the peritoneal fluid, and in the subcutaneous tissue. Twenty mice with primary tumors showed no infection, and their tumors also were free from spirochetes. This prevalence among normal mice, together with the fact that the spirochetes can be transmitted experimentally, strengthens the view that they are not an etiological factor in tumor formation, but only an accidental contamination.

Deetjen, who favored this view, attempted to separate the spirochetes from the tumor, but was not successful. I have also tried to do this with intravenous injections of Diarsenol. The idea was to kill all spirochetes at once by a sufficient intravenous dose and so to make the tumor free from spirochetes—that is, to attempt Ehrlich's *therapia sterilisans magna*. The attempt was successful. Twenty-six immune and non-immune inoculated mice were injected in the tail vein with a 1 per cent Diarsenol solution, 0.2 to 0.26 cc. being found sufficient; higher doses usually caused severe collapse. All the mice treated in this way remained permanently free from spirochetes. No effect upon the existing tumors could be observed, for they continued to grow as before; but they were all free from spirochetes. These tumors, made sterile by Diarsenol, were now inoculated into 43 spirochete-free mice. Twenty-five (58 per cent) of these developed tumors, but none of the mice and none of the tumors contained spirochetes. Tumors growing in this batch of mice were again transplanted into 44 spirochete-free mice, of which 29 (66 per cent) proved susceptible. All mice in this second generation after the Diarsenol injection remained free from spirochetes, as did the tumors. These experiments prove definitely that there is no etiological relation between spirochetes and tumor.

We have apparently to deal with a widespread spirochetal infection among mice. When a primary or transplanted tumor develops in an infected mouse, the spirochetes living in all the body fluids are transported in larger or smaller numbers, according to the blood supply, into the tumor. This explains their great prevalence at the margin, where there are most blood-vessels. They are then inoculated together with the tumor into other mice, and as all mice are susceptible they give rise to a general spirochetosis.

That this actually does happen is proved by the following experiment. Fifteen mice were infected by the intraperitoneal injection of blood and peritoneal fluid containing spirochetes, and four days later inoculated with one of those tumors which had remained free of spirochetes through two generations by reason of the Diarsenol treatment previously referred to. Four mice were lost before a secondary tumor had time to appear, but six (53 per cent) of the remaining mice developed growths. As expected, all these mice showed spirochetes, and the organisms could be found also in all the tumors. These tumors, now containing spirochetes, were inoculated into 13 spirochete-free mice, with the result that 9 became infected and 6 (46 per cent) developed tumors; of these 6 growths, the 4 that could be examined showed spirochetes.

It is interesting to note the change in the virulence of the tumor during these experiments. The untreated tumor gives about 80 per cent takes in our routine transplantations; the spirochete-free tumor taken from the Diarsenol mice produced, in the first generation, only 58 per cent, and in the second 66 per cent successful inoculations. After the tumor had been reinfected with spirochetes, its virulence decreased to 54 per cent in the first, and 46 per cent in the second generation. The tumor, therefore, had lost almost half its virulence, probably because the vitality of its cells had been impaired by the Diarsenol treatment of their host and the reinfection with spirochetes. But experiments on a much larger basis are of course necessary, to throw more light on this question.

SUMMARY

1. All our mice inoculated with tumor R. I. 33 had spirochetes in the blood, peritoneal and pleural fluids, in the subcutaneous tissue, and in the tumors. The spirochetes can be transmitted to other mice intraperitoneally or subcutaneously without difficulty.

2. A similar spirochete can also be found in almost 2 per cent of normal mice.

3. The spirochetes are not an etiological agent, but merely a contamination of this tumor.

4. The spirochetes can be separated from the tumor and re-introduced into it.

5. The virulence of the tumor has decreased from 80 per cent to 46 per cent "takes" during these experiments.

Dr. Clowes: It is interesting to note that the Diarsenol used in the experiments reported by Dr. Mueller was employed in a dosage considerably lower than that required to kill the mice. It appears to possess a remarkable penetrating power. The fall in the virulence of the tumor corresponds with our observations, made some years ago, regarding the influence of potassium cyanide in destroying bacteria in tumors. It was found possible to destroy bacteria and yet leave the tumor sterile, so that it could be transplanted; but its virulence was greatly impaired.

THE EFFECTS OF X-RAY IRRADIATION ON LIVING CARCINOMA AND SARCOMA CELLS IN TISSUE CULTURES IN VITRO¹

NORIYOSHI KIMURA

*From the Pathological Laboratory of the University of Chicago and the Otho S. A.
Sprague Memorial Institute*

Received for publication, January 28, 1918

During the years which have passed since the method of tissue culture in vitro was commenced by Harrison, and modified by Carrel and Burrows (4), very little attention has been paid the investigation of the biological effects of *x*-rays upon cells growing in culture in vitro.

Contamin (7) exposed mouse carcinoma to *x*-rays under various experimental conditions. From the result of a number of his observations, he came to the conclusion that the younger the tumors, the more sensitive they were to *x*-rays, and that the disappearance of a large tumor under *x*-rays caused the death of the animal, probably by intoxication. In other experiments made by Contamin (18) with Nogier and Jaubert de Beaujeu, extirpated mouse carcinoma was exposed to *x*-rays and then inoculated into normal mice. They concluded that the action of *x*-rays on extirpated tumor cells hinders their subsequent growth in the animal body.

Clunet (5) and Raulot-Lapointe treated malignant tumors in situ with *x*-rays and studied them histologically at various stages of the treatment. They found that the squamous carcinoma cells in the human subject finally disappeared, passing through five successive stages from the latent phase to the formation of the connective tissue scar. With sarcomatous growths, Clunet and Raulot-Lapointe found that the latent phase was much shorter than in the other types of malignant growths.

¹The author has not read the proof of this article.

Wedd and Russ (31) reported a series of experiments in which a transplantable mouse carcinoma was removed from the animal in which it had grown, kept between mica sheets during the exposure to radium rays, and then inoculated into normal mice. It was found that no growth resulted from grafts which were exposed a sufficient length of time.

Russ and H. Chambers (25) reported an observation made with Jensen's sarcoma. The authors concluded that the tumor cells irradiated by β -rays of radium (1.63 mgm. per square centimeter for ninety-six minutes) or radium emanation of 0.53 millicuries per cubic centimeter for forty-five minutes, did not produce tumors after inoculation into normal mice, though they showed no histological changes in the cells.

Wassermann (30) exposed extirpated cancerous tissue to radium rays from mesothorium, and then inoculated it into animals, no tumor resulting. He supposed that the multiplication and cell division were affected by the rays while the nutrition of the cells remained uninfluenced, though he did not try cultures in vitro.

C. Price Jones (12) exposed mouse carcinoma and mouse sarcoma to radium rays, made tissue cultures of them, and found that the mitotic division of cells was inhibited, though their spreading growth was not retarded.

Prime (23) has recently worked in the same direction, reporting that a carefully measured amount of radium rays injured the power of mitotic cell division in the tissue, and that the inoculation of a sufficiently irradiated tumor produced no growths in mice.

In all these articles, however, no observations seem to have been made on the effects of x -rays upon living cells growing in plasma outside of the body. The present experiments were undertaken, therefore, to clear up the question of the biological effect of x -rays on tumor cells, and to determine what dose of x -rays would render tumor cells incapable of producing tumors when subsequently inoculated into animals.

PREPARATION OF THE CULTURE MEDIA

A small glass tube (*A* in text-fig. 1) was coated thickly with paraffin and inserted into an aluminum centrifuge tube (*B* in



Text fig. 1. Tube in which plasma is obtained.

text-fig. 1) stopped by means of a cork. The space between tubes *A* and *B* was filled with ice and salt mixture, and the whole apparatus kept in a glass with ice mixture until ready for use.

Under ether anesthesia the carotid artery of a guinea-pig was exposed about 2 to 3 cm. The distal part was ligatured by a thread while the proximal part was fixed by a small artery clamp to stop the blood stream. Proximal to the ligature the artery was pinched by a small clamp without narrowing the lumen.

Between this and the ligature the artery was cut off sharply. After the proximal clamp had been released, the blood was allowed to flow directly into the tube A, which was kept cool in ice mixture. When the blood was filled up to one-third the height of the tube A, the artery was clamped again. The tube with blood was promptly and powerfully centrifugalized for three minutes. The supernatant plasma (text-fig. 1) was separated by a small pipette into four or five small tubes, which were coated thickly with paraffin and prepared in ice mixture. It was necessary neither to keep the pipette cool nor to coat it with paraffin. These tubes which held the plasma were plugged with sterilized cotton and covered with tinfoil in order to protect them from contamination or drying, and preserved in a frozen condition in a Universal jar filled with ice mixture. The greatest difficulties encountered were obtaining a sufficient amount of mouse blood and keeping the plasma from coagulating. The mouse was prepared by the removal of the hair from the throat region, and the skin rendered sterile by iodine. The skin over the thyroid region was picked up with a forceps and cut off with scissors. The thyroid gland was picked up and bluntly loosened from the under layer without injuring the tissues. The carotid artery appeared on both sides in the bottom of the space made by picking up the gland. After the artery had been cut, the escaping blood was collected in the paraffin coated tube (text fig. 1) in ice mixture. In the same manner as described already, the tube was immediately centrifugalized for three minutes. The supernatant plasma was drawn up and put into cold tubes in ice mixture.

It was found very easy to obtain chicken plasma. A heavy syringe needle, sterilized by boiling, was inserted directly into the wing vein and the blood was collected drop by drop in an ice cold

paraffin coated tube, and centrifugalized in the same way as above. The supernatant plasma was drawn off into tubes.

The plasma thus obtained from guinea-pigs, mice, and chickens, did not lose its coagulability for about ten to fourteen days as it was kept in ice mixture, and remained fluid for hours during each series of experiments, when it was kept cool in ice, and at no time did coagulation take place before the completion of the experiment. One tube containing 0.2 to 0.3 cc. of plasma was enough for each series of experiments.

MATERIAL AND TECHNIQUE OF CULTURE

The tumors used were mouse carcinoma R.T. 33, which had been propagated for three years at the State Institute for the Study of Malignant Disease, Buffalo, New York. These tumors, for which I am indebted to Dr. Gaylord, showed a type of adenocarcinoma (fig. 2) and with the mice which I had inoculated gave "takes" in about 90 per cent of implants. Another tumor, for which I am indebted to Dr. F. C. Wood of the Crocker Fund, was the Ehrlich mouse sarcoma (fig. 3), which had been under observation for some years in the Crocker Laboratory in New York, and gave approximately 100 per cent of successful inoculations in Chicago mice.

The tumors, after reaching a full growth, were cut out strictly aseptically in Ringer's solution and cut into many pieces of equal size. They were then put into three or four small sterilized glass tubes with a diameter of 1 cc. and 1.5 cc. in depth. Each of these glass tubes was stopped with a cork pushed in over a sheet of sterile Japanese paper in order to prevent any contamination that might occur during the exposure of the *x*-rays. After the removal of the cork stopper, the pieces of tumor, which were kept in these tubes with Ringer's solution, were exposed to *x*-rays merely through the layer of Japanese paper at a distance of 2 cm. from the *x*-ray bulb. In this manner each piece of tumor was exposed for a certain required length of time without other obstacles between the bulb and the tumor pieces. Another factor in favor of this method was the fact that the tissues did

not begin to grow while exposed to the ray as was found to be the case when they were planted first into the plasma and then exposed.

The ray used for this series of experiments was of a moderately soft type, the spark gaps ranging from 4 to 8 cm.; the length of time for exposure varied from five to thirty-six minutes. The amount of the effective ray was measured by Hampson's radiometer. The initial tint is the color of the unexposed pastille and the sixteenth change represents the browner shade of color, equivalent to the maximum or B tint of the Sabouraud's pastille. The terms E.1, E.4, E.8, or E.12 in the following experiments indicate that the Hampson's pastille used showed no. 1, no. 4, no. 8 or no. 12 tint, that is, equivalent to a dose $\frac{1}{16}$, $\frac{1}{4}$, $\frac{1}{2}$, or $\frac{3}{4}$ of Sabouraud's B tint.

The irradiated tissue was removed to a watch glass with Ringer's solution and was cut into fine fragments. Each fragment was transferred to a cover glass by a small pipette provided with suction bulb. The excess Ringer's solution was sucked up with the same pipette. Some small drops of plasma were added immediately to the tissue fragment, then some drops of mouse serum with Ringer's solution were added. The plasma and serum were mixed up and spread around the fragment with a pointed cataract knife. The cover slip, which previously was ringed with vaseline on all its edges, was then inverted over a fairly deep hollow ground slide. The cover slip was then sealed with molten paraffin around its edges. An equal number of control cultures from an irradiated tissue was made in each series. The slide preparations were incubated at 37°C. and microscopic observation was made every twelve to twenty-four hours.

To obtain the stained specimen the cover slips, mounted with growing culture, were put into 10 per cent formalin for twenty-four hours or more, then they were washed in water for one hour and stained with diluted Delafield's haematoxylin for one to two hours, and decolorized for about thirty minutes in water, to which a few drops of diluted hydrochloric acid was added. The specimens were washed again in water until a violet blue color developed. It is important to decolorize the over-stained plasma and

tissue as much as possible, otherwise no good specimen was obtained.

The tissue piece irradiated with certain required doses of x-ray in Ringer's solution was divided into many fragments, each of about 20 mgm., and these were inoculated into the right axilla of a number of normal mice. An equal number of tissue fragments from the control tissue were inoculated into the other axilla of the same mice. The observations respecting the rate and course of the grafts planted into the animals follows.

The culture media used for the experiments were: Mouse plasma, guinea-pig plasma, chicken plasma, guinea-pig plasma plus mouse serum diluted with Ringer's fluid, chicken plasma plus mouse serum diluted with Ringer's fluid.

In comparing all these media used for the culture of the tumor, it was found that the mouse plasma (homogenous and autogenous) or a mixture of guinea-pig plasma and mouse serum diluted with Ringer's solution were most satisfactory as media for the culture of the tumors. There was no noticeable difference in either case, whether the mouse plasma or the guinea-pig plasma with diluted mouse serum was used. Because the guinea-pig plasma can be obtained much more easily and in greater quantity than mouse plasma, most of the cultures, except a few specimens, were made with guinea-pig plasma, to which was added the mouse serum and Ringer's solution.

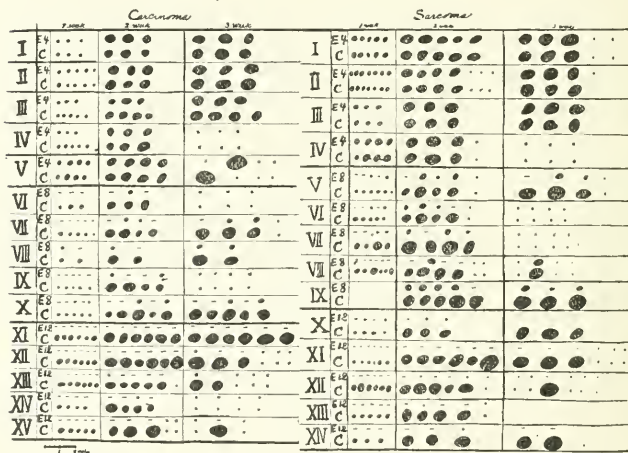
RESULTS OF EXPERIMENTS

Mouse carcinoma

Mouse carcinoma, cultivated under the conditions described above in respect to technique, showed but very little growth the first twenty-four hours in most cases of all series, both in the control and in the exposed tissue. After twenty-four hours of incubation, the original fragment of tissue became thinner and somewhat translucent, especially on the edge of the fragments, from which the cells spread out into the plasma media. The cells did not migrate separately into the media, but merely formed cell groups composed of a number of cells. The boundaries of each cell were indistinct.

Many of the cells on the advancing edge formed pseudopodia. There were many cells of different morphological type which supposedly originated from the stroma. Cells of this type were present in some specimens, while others showed no such type of cells.

Most cultures reached the maximum growth in forty-eight to ninety-six hours after incubation. Karyokinetic figures were observed in the cells in the growing zone in stained specimens



Text fig. 6. No difference between irradiated (E.4 dose) and control tumor, after inoculation.

from the control tissue (fig. 4). The specimen from the tumor piece exposed to E.1 of rays, showed no difference from the control in growth in the plasma media during the whole observation, and mitotic figures of growing cells were seen frequently in the stained preparations in all stages. Both the tissues, control and irradiated, produced good sized tumors after inoculation into normal mice.

In cultures from the tissue exposed to E.4 of the ray, the proliferation of cells was just as extensive as in the specimen made from control tissue (figs. 9, 10), while the spreading-out of the thread-like cells, which supposedly originated from stroma, showed itself a little more active than in the control. The mitotic figures were found as abundant as in control cultures (fig. 5).

No difference was seen in speed and rate of growing tumors, produced by inoculation of either the treated or the control piece, as is shown by the preceding chart (text-fig. 6) and by table 1.

TABLE 1
Carcinoma

	NUMBER OF "TAKES"		PER CENT	NUMBER OF "TAKES"		PER CENT	NUMBER OF "TAKES"		PER CENT
	E.4	Control		E.8	Control		E.12	Control	
First week.....	19	19	100	3	19	16.0	0	26	0
Second week.....	16	16	100	8	18	44.5	0	24	0
Third week.....	10	10	100	6	10	60.0	0	12	0

In the unstained culture, when E.8 doses of *x*-ray were given, the proliferation of cells growing in groups showed almost the same extension as did the specimen of control tissue (fig. 11). In some specimens, however, the cells of the stroma type, which spread out like fibrillae into the plasma media, seemed more vigorous than those in the control specimens. But in the stained specimen a few mitotic figures were found in most of the specimens cultivated from the irradiated tissue (fig. 7), while many more mitotic figures appeared in the control specimen (fig. 4). After the inoculation into mice, it was found that the treated tissue gave "takes" of 16 per cent in the first, 44.5 per cent in the second, and 60 per cent in the third week, according to table I, while the control tissue produced tumors in 100 per cent of the grafts.

All the tumors grown in mice from the treated cells, however, reached only one-third of the size of those from the control inoculations.

In the cultures of the tissue which was exposed to E.12 of the active ray, it was found that after forty-eight hours there was still a marked outgrowth of cells (fig. 12). The same results were found in the control specimen. None of the stained specimens of the exposed tissues showed mitotic figures in growing cells (fig. 8).

The inoculation of the treated tissue into mice produced in the first week no tumor in 27 cases in 6 series. In the second and third weeks two nodules were developed to the size of a rice grain; they, however, did not grow further, but disappeared.

Mouse sarcoma

In the cultures made in guinea-pig plasma diluted with mouse serum, the margin of the fragment became gradually sharp and opaque. In five to twelve hours a few round cells had emigrated into the plasma media and a few irregular cells began to grow out radially from the edges of the fragment, which began to have a serrated appearance. The individual cells formed many pseudopodia, which were seen especially in the advancing side of the cell body. The number of cells emigrating into the media increased more rapidly than those in the culture of carcinoma tissue. After forty-eight hours the original fragment became more translucent than it was before, and it was surrounded with thick layers of growing cells, which were rich in protoplasm. When the stained specimens were fixed, at the end of twenty-four to seventy-two hours, they exhibited the presence of numerous mitotic figures in the outgrowing cells (fig. 13).

The specimen from the tissue exposed to E.1 of x -ray showed no difference in respect to the rate of growth from that of the control tissue (fig. 17). The inoculation of the treated piece into mice produced tumors as large as those of the control tissue.

In the culture of the tissue exposed to E.4, the proliferation of cells was found to be more vigorous than that in the control cultures (fig. 18). In the stained preparations, however, the number of mitotic figures of cells was about equal to that in the control specimen (fig. 14). After inoculation into mice, the

tumors produced from irradiated cells in the first week seemed to grow a little faster than those in controls; but in the second and the third week there was no difference either in speed or size, and the inoculation gave "takes" of 100 per cent.

In the unstained cultures from the tissue which was exposed to E.8, the proliferation of cells and the area of growth were similar to the proliferation in the control specimen (fig. 19). But the mitotic figures in the stained specimen diminished greatly in number compared with those in the control specimen (fig. 15). In the first week after inoculation, the grafts gave "takes" of 35 per cent, 62 per cent in the second, and 85.7 per cent in the third week. These tumors, however, developed only to one-third the size of those in the controls (text-fig. 6).

TABLE 2

Sarcoma

	NUMBER OF "TAKES"		PER CENT	NUMBER OF "TAKES"		PER CENT	NUMBER OF "TAKES"		PER CENT
	E.4	Control		E.8	Control		E.12	Control	
First week.....	19	19	100	7	20	35.0	0	24	0
Second week.....	15	15	100	10	16	62.5	0	21	0
Third week.....	9	9	100	6	7	85.7	0	9	0

In the cultures made from the tissue which had been irradiated to E.12, it was found that there was still a marked outgrowth of cells as well as in the control specimen (fig. 20). No mitotic figures, however, were found in the stained specimen (fig. 16). None of the inoculated grafts into 23 mice in 5 series produced any tumor the first week. In the second week there were found 4 hardly perceptible nodules in 21 mice. These, however, did not grow further and disappeared entirely in the third week, as is shown in text-fig. 6 and table 2.

DISCUSSION

In accordance with the results of the experiments described above, the rate of the cell growth in cultures of mouse carcinoma and mouse sarcoma was, after forty-eight to ninety-six hours'

incubation, nearly equal in both the control and the exposed tissues which were *x*-rayed to E.1–E.12 (figs. 9, 10, 11, 12, 17, 18, 19, 20). The sarcoma, as compared with the carcinoma, however, was always superior in its growth in culture media. After an exposure of E.8, the number of the mitotic figures in the culture of growing tumor cells was diminished to a minimum of 2 to 4 in carcinoma and 2 to 13 in sarcoma cultures. An exposure, however, of E.12, entirely inhibited the mitotic division of cells, and they were never found in the stained specimen, either in carcinoma or in sarcoma (figs. 8, 16). See table 3.

By an exposure of E.4, the sarcoma not only remained without injury to the power of proliferation by mitotic cell division, but the exposed tissue produced tumors in the first week after inoculation —i.e., somewhat earlier than the control tissue did (text-fig. 6). This phenomenon was due to the action of *x*-rays, to which the tissue was exposed. It seems that the *x*-ray in this dose acted upon the tissue as a stimulation and temporarily raised the metabolism of the tumor cells. Consequently the cells in the exposed tissue were stimulated to grow more quickly the first week after inoculation than those in the control.

In connection with the process of oxidation in the tumor tissue, I tried some experiments hoping that the effects of *x*-ray on the living cells might be explained to some extent. The tumor pieces were exposed to *x*-ray of various doses varying from E.4 to E.12. One piece of control was put in the chamber of one side of Dr. Tashiro's biometer and an irradiated piece in the other chamber of the other side.

Observation was made as to the quantitative difference of CO₂ production in both chambers. The results of these experiments are shown in the record of tables 4 and 5.

In the experiments with the tumor pieces, both in carcinoma and sarcoma, which were exposed to E.4 of *x*-ray, carbon dioxide production began to appear in ten minutes after the arrangement. The quantity of precipitation by barium hydroxide in the chamber which contained the exposed tissue was greater than that in the chamber which had the control tissue.

TABLE 3

SPECIMEN	NUMBER OF FIGURES	AMOUNT OF X-RAY	LENGTH OF TIME	STAINED SPECIMEN	
				Growth in culture	Number of mitotic figures
Carcinoma					
111 4 31 102	4, 9	Control	minutes	Fair Fair Slight Slight	7 15 8 16
110 104 101 103	10, 5	E.4	12	Fair Fair Slight Slight	6 8 4 7
18 100 35 28	11	E.8	24	Fair Slight Fair Slight	2 3 4 4
5 33 34 107	8, 12	E.12	36	Fair Slight Slight Fair	0 0 0 0
Sarcoma					
1 95 105 106	13	Control		Fair Fair Fair Fair	21 40 Numerous Numerous
43 17 52 15	14, 18	E.4	12	Fair Fair Fair Fair	40 25 31 27
11 98 94 50	15, 19	E.8	24	Fair Fair Fair Fair	10 6 13 2
96 41 46 53	16, 20	E.12	36	Fair Fair Fair Fair	0 0 0 0

On the contrary, in the experiments made with the tissue exposed to E.12 of x-ray, the production of carbon dioxide was less than that of the control.

In the unstained specimen cultured from tissue which was exposed to E.4, the outgrowth of cells seemed somewhat more

TABLE 4
Carcinoma

CO ₂ COMPARED			CO ₂ COMPARED		
Time	E.12	Control	Time	E.4	Control
	*29 mgm	*27 mgm.		*75 mgm.	*75 mgm.
4.42	-----	-----	2.19	-----	-----
4.50	-----	= -----	2.29	-----	> -----
7.00	-----	< -----	2.39	-----	> -----
	++	+++	2.49	-----	> -----
			2.59	+++--	> -----+
	52 mgm.	50 mgm.		75 mgm.	75 mgm.
7.50	-----	< -----	2.35	-----	-----
7.55	-----	< -----	2.45	-----	> -----
8.00	-----	< -----	2.55	-----	> -----
8.10	-----	< -----	3.05	-----	> -----
	++	+++	3.15	-----	> -----
			3.35	-----	> -----
				++	+
	57 mgm.	55 mgm.		63 mgm.	64 mgm.
7.22	-----	-----	2.13	-----	-----
7.32	-----	-----	2.23	-----	> -----
8.12	-----	< -----	2.33	-----	> -----
8.20	-----	< -----	2.43	-----	> -----
8.40	-----	< -----	3.13	-----	> -----
	++	+++		+++	++

* The figures are the weights of the pieces of tumor tissue used in each experiment. No quantitative determination of the amount of CO₂ produced was made, but the relative amounts produced by irradiated tumor and control are indicated by the > sign.

extensive than the outgrowth of the control culture, both in sarcoma and carcinoma. But in the stained specimens no noteworthy difference in number of the mitotic figures was seen. This stimulating effect on the sarcoma tissue was more prominent than the effect shown in the culture of the carcinoma. A some-

what similar difference of the stimulating effect of x -ray E.4 on the tissues appeared prominently in the inoculation experiments of sarcoma, but the carcinoma was not much stimulated by the rays and consequently the power of proliferation was diminished

TABLE 5

Sarcoma

CO ₂ COMPARED			CO ₂ COMPARED		
Time	E.12	Control	Time	E.4	Control
	*74 mgm.	*70 mgm.		*132 mgm.	*132 mgm.
2.25	-----	-----	3.43	-----	-----
2.40	-----	= -----	3.53	-----	> -----
2.45	-----	< -----	4.03	-----	> -----
3.00	-----	< -----	4.43	-----	> -----
3.10	-----	< -----			
3.25	-----	< -----		++++	+++
	++	+++			
	22 mgm.	16 mgm.		132 mgm.	132 mgm.
2.36	-----	-----	4.13	-----	-----
2.46	-----	< -----	4.23	-----	> -----
2.56	-----	< -----	4.33	-----	> -----
	+	++	5.13	-----	> -----
				++++	+++
	48 mgm.	47 mgm.		85 mgm.	85 mgm.
3.38	-----	-----	6.33	-----	-----
3.48	-----	< -----	6.43	-----	> -----
3.58	-----	< -----	6.53	-----	> -----
4.08	-----	< -----	7.03	-----	> -----
4.18	-----	< -----			
4.28	-----	< -----		++	+
	+	+++			

* The figures are the weights of the pieces of tumor tissue used in each experiment. No quantitative determination of the amount of CO₂ produced was made, but the relative amounts produced by irradiated tumor and control are indicated by the > sign.

gradually without any preliminary stimulation (text-fig. 6 and table 4).

The fact that the number of mitotic figures in culture and the quickness of growth of the grafts inoculated into mice increased to some extent when the tissues previously were exposed to E.4

of rays, and the fact that both decreased gradually when the tissues were irradiated to E.8-E.12, coincide with the results of the experiments on the oxidation of the tumor tissues.

For the explanation of the presence of only a few mitotic figures in the culture and retardation of the growth of the grafts when the tissue was exposed to E.8, the reports made by Bordier and others are to be taken into consideration. We know that the younger the cell generation the greater the radio-sensibility of the living protoplasm; and consequently the younger neoplastic cells are most sensitive, while the cells in the latent stage are less sensitive to the x -ray action. Hence the mitotic figures seen in the tissues that had been exposed to E.8 supposedly originated from some resting cells in the fragments, in which they remained without an intensive effect of x -ray and produced the further division in culture in vitro or developed to a tumor in mice, though their mitotic proliferation was greatly retarded.

On the contrary, all the cells in the specimen exposed to E.12 were sufficiently damaged by the ray action, and consequently the dividing process of the cell chromosomes had ceased, and the fragments did not grow any more to a tumor in mice.

CONCLUSIONS

1. The mouse carcinoma and sarcoma grow as well in guinea-pig plasma to which has been added mouse serum diluted with Ringer's solution, as in mouse plasma itself.

2. The culture growths of carcinoma and sarcoma from mice showed each the characteristics of the original tissues. Sarcoma produced a radial outgrowth spreading widely into the plasma media, while the carcinoma cells grew continuously into the media, as cell groups, from the edges of the fragments.

3. The outspreading growth of cells in culture, both sarcoma and carcinoma, was not stopped by x -ray action varying from E.4 to E.12. The mitotic figures of cells were limited to a minimum after an exposure of E.8 (one-half dose of Sabouraud's B tint). After exposure to E.12 (three-fourth dose of Sabouraud's B tint), however, they disappeared entirely, and the treated tissue produced no tumor when inoculated into mice.

4. The growing power of sarcoma after E.4 exposure was stimulated to some extent, while carcinoma was not appreciably influenced. An exposure of tissues to E.12, both sarcoma and carcinoma, stopped the growing power of these tissues when inoculated into mice, and eliminated the process of mitotic division of cells.

5. The process of oxidation of tissues, both sarcoma and carcinoma, was stimulated by the x -ray action of E.4 and retarded by exposure to E.12 of the ray.

REFERENCES

- (1) APOLANT: Deutsch. med. Wehnschr., 1904, xxx, 454.
- (2) BORDIER: Fortschr. a. d. Geb. d. Röntgenstrahlen, 1913, xx, 202.
- (3) BECKTON: Arch. Middlesex Hosp., 1914, xxxiii, 123.
- (4) CARREL AND BURROWS: Jour. Exper. Med., 1911, xiii, 387 and 571.
- (5) CLUNET AND RAULOT-LAPOINTE: Recherches Expérimentales sur les Tumeurs Malignes, Paris, 1910.
- (6) COLWELL AND RUSS: Radium, X-rays and the Living Cell, London, 1915.
- (7) CONTAMIN: Compt. rend. Acad. d. sc., 1910, cl, 1537.
- (8) EBELING: Jour. Exper. Med., 1914, xx, 130.
- (9) HEIMANN: Berl. klin. Wehnschr., 1914, li, 1516.
- (10) HEINEKE: München. med. Wehnschr., 1913, lx, 2657.
- (11) INGBRIGSTEN: Jour. Exper. Med., 1912, xvi, 421.
- (12) JONES AND MOTTRAM: Arch. Middlesex Hosp., 1914, xxxiii, 21.
- (13) LAMBERT AND HANES: Jour. Exper. Med., 1911, xiii, 495; 1911, xiv, 129; 1913, xviii, 406; 1914, xix, 398.
- (14) LAMBERT: Anat. Rec., 1912, vi, 91.
- (15) LAMBERT AND HANES: Virchows Arch., 1913, cexi, 89.
- (16) LAMBERT: Jour. Cancer Research, 1916, i, 169.
- (17) LOEB: Anat. Rec., 1912, vi, 109.
- (18) CONTAMIN, NOGIER, AND JAUBERT DE BEAUJEU: Compt. rend. Acad. d. sc., 1909, cxlix, 1398.
- (19) OBERNDORFER: Zentralbl. f. allg. Path. u. path. Anat., 1914, xxv, 411.
- (20) PACKARD: Jour. Exper. Zool., 1915, xix, 323; 1915, xxi, 199.
- (21) PETRY: Biochem. Ztschr., 1913, lvi, 341.
- (22) PENTIMALLI: Ziegler's Beiträge, 1914, lix, 674.
- (23) PRIME: Jour. Cancer Research, 1917, ii, 107.
- (24) RICHARDS: Am. Jour. Roentgenol., 1915, ii, 908.
- (25) RUSS AND CHAMBERS: Proc. Roy. Soc. Med., London, 1913, vi, 62-68.
- (26) SCHWARZ: München. med. Wehnschr., 1913, lx, 2165.
- (27) TASHIRO: Chemical Signs of Life, University of Chicago Press, 1917.
- (28) WALTON: Jour. Exper. Med., 1914, xix, 121; 1914, xx, 554.
- (29) WALTON: Proc. Roy. Soc., London, 1914, lxxxvii, Series B, 452.
- (30) WASSERMANN: Deutsch. med. Wehnschr., 1914, xl, 524.
- (31) WEDD AND RUSS: Jour. Path. and Bacteriol., 1912, xvii, 1.
- (32) WERMEL: München. med. Wehnschr., 1914, lxi, 299.

PLATE 1

FIG. 2. Stained section of original carcinoma, many mitotic figures. X 275.

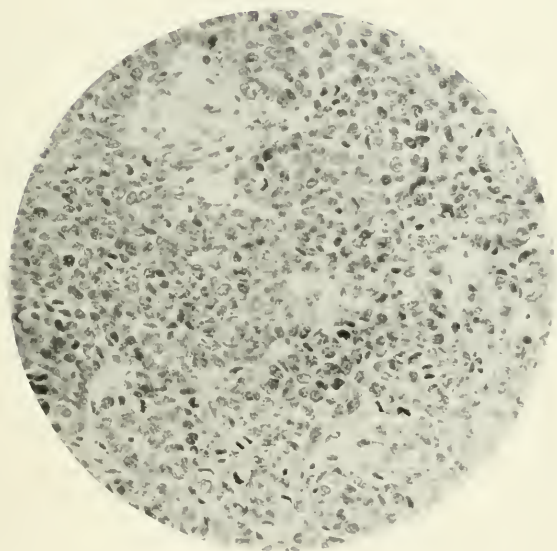


PLATE 2

Fig. 3. Stained section of original sarcoma, many mitotic figures. $\times 275$.

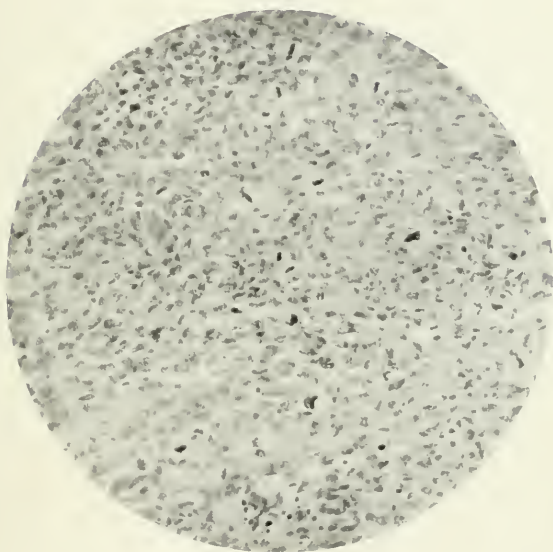


PLATE 3

Fig. 4. Tissue culture, control, carcinoma, 2 mitotic figures. $\times 275$.

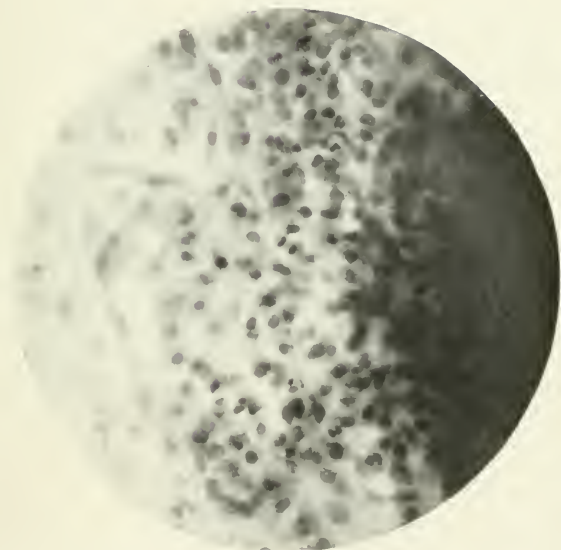


PLATE 4

Fig. 5. Tissue culture, experiment 4, carcinoma, 1 mitotic figure. $\times 275$.

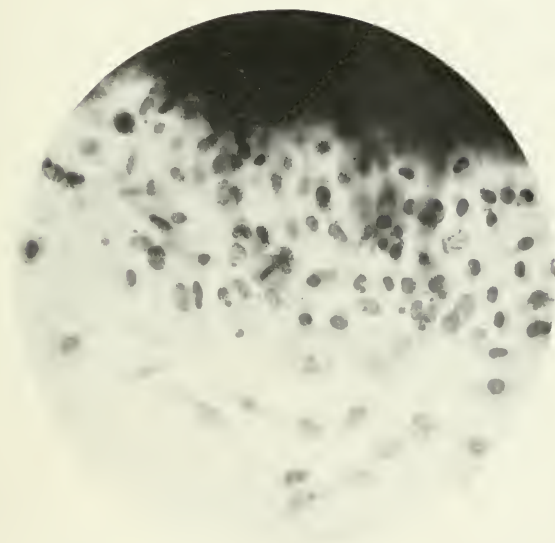


PLATE 5

Fig. 7. Tissue culture, experiment 8, carcinoma, 1 mitotic figure. $\times 275$

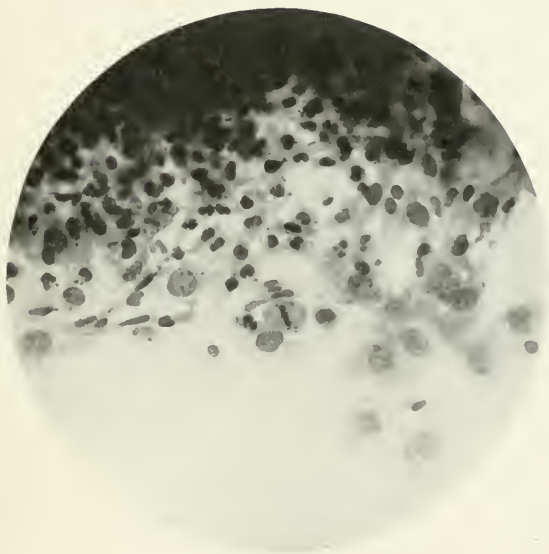


PLATE 6

Fig. 8. Tissue culture, experiment 12, carcinoma, no mitotic figure. $\times 275$

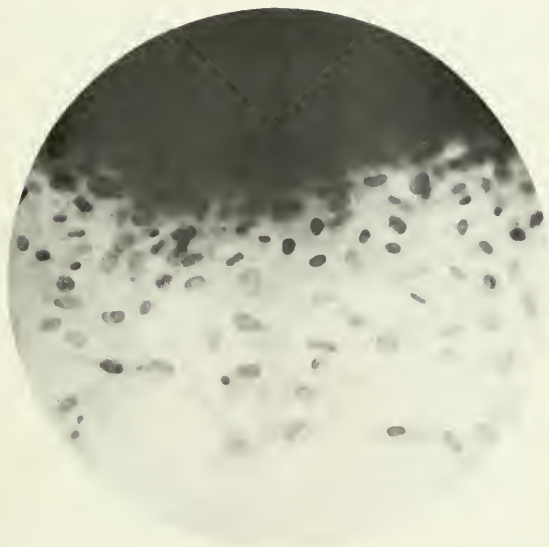


PLATE 7

Fig. 9. Tissue culture, control, carcinoma. $\times 37$.

Fig. 10. Tissue culture, experiment 4, carcinoma. $\times 37$.

Fig. 11. Tissue culture, experiment 8, carcinoma. $\times 37$.

Fig. 12. Tissue culture, experiment 12, carcinoma. $\times 37$.

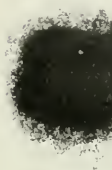


FIG. 9

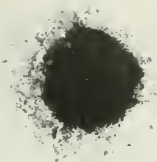


FIG. 10



FIG. 11



FIG. 12

PLATE S

Fig. 13. Tissue culture, control, sarcoma, 2 mitotic figures. $\times 275$.

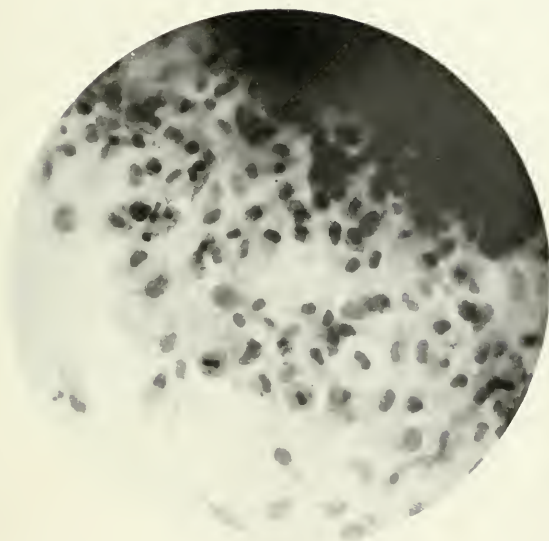


PLATE 9

Fig. 14. Tissue culture, experiment 4, sarcoma, 4 mitotic figures. $\times 275$.

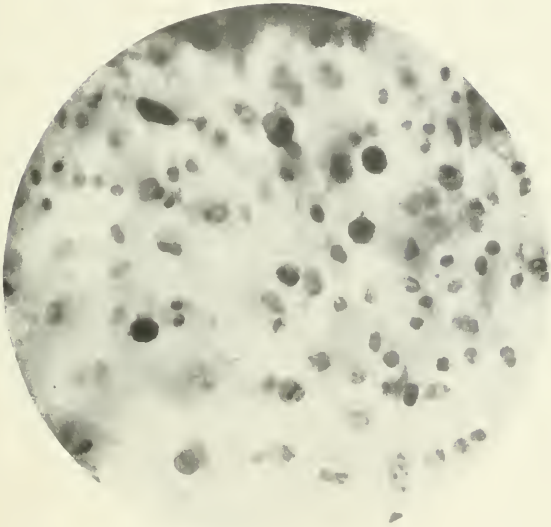


PLATE 10

Fig. 15. Tissue culture, experiment 8, sarcoma, 1 mitotic figure. $\times 275$.

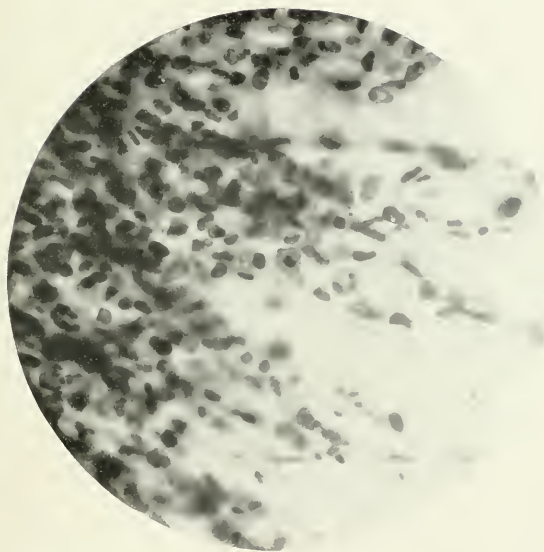


PLATE 11

Fig. 16. Tissue culture, experiment 12, sarcoma, no mitotic figure. $\times 275$.

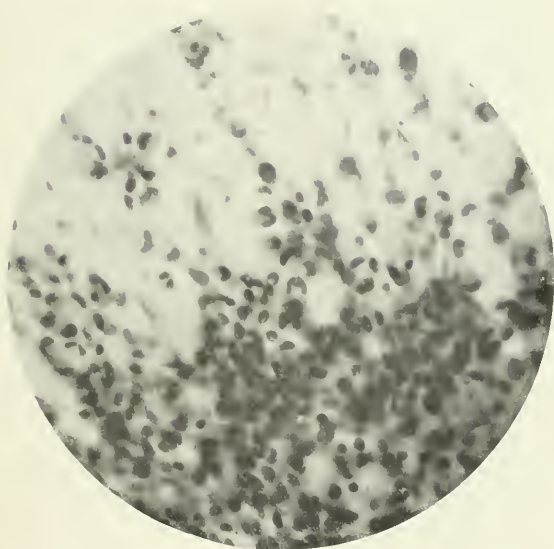


PLATE 12

Fig. 17. Tissue culture, control, sarcoma. $\times 37$.

Fig. 18. Tissue culture, experiment 4, sarcoma. $\times 37$.

Fig. 19. Tissue culture, experiment 8, sarcoma. $\times 37$.

Fig. 20. Tissue culture, experiment 12, sarcoma. $\times 37$.



FIG. 17

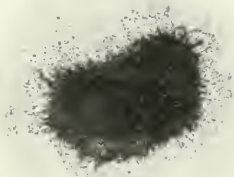


FIG. 18

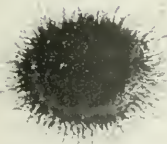


FIG. 19



FIG. 20



FURTHER INVESTIGATIONS ON THE ORIGIN OF TUMORS IN MICE

IV. THE TUMOR INCIDENCE IN LATER GENERATIONS OF STRAINS WITH OBSERVED TUMOR RATE.

A. E. C. LATHROP AND LEO LOEB

*(From the Department of Comparative Pathology of Washington University School
of Medicine, St. Louis.)*

Received for publication, January 30, 1918

In previous publications we reported on the difference in the tumor rate which existed in different strains of mice and on the hereditary transmission of this tumor incidence in the simple strains and in the hybrids of different strains. We also gave special attention to the tumor age in the various strains (1). It seemed to us to be of great interest to follow the fate of these strains in order to obtain further insight into the factors which determine the occurrence and origin of tumors in mice. We know that in the course of long continued inbreeding of certain families, without special precautions being taken changes do often occur in the later generations. How far do such changes influence the tumor rate? While our observations are not yet complete, and while further work will be necessary before all the factors involved can be considered satisfactorily analysed, we believe that our present data, which are based on a very extensive material, throw some light on these problems. We shall first consider the various strains, comparing the behavior in the later and in the earlier generations. As to the arrangement of our data and the meaning of the figures, we refer to the explanations given in our earlier papers.

STRAIN LONDON

We have considered in our former paper the F_1 , F_2 , and F_3 generations of this strain. In those generations the tumor incidence and age were as follows:

<i>Without tumor</i>	<i>With tumor</i>
73% (41% I 29% II 30% III)	27% (27% I 43% II 30% III)

The mice belonged to the second age class.

Since then the following groups of *London* mice have been observed:

<i>Without tumor</i>	<i>With tumor</i>
F_3 8 (2 I 1 II 5 III)	
F_4 35 (5 I 13 II 17 III)	15 (4 I 6 II 5 III)
70% (15% I 37% II 48% III)	30% (26% I 40% II 34% III)
F_5 68 (27 I 23 II 18 III)	25 (12 I 13 II)
73% (39% I 34% II 27% III)	27% (48% I 52% II)
F_6 39 (12 I 21 II 6 III)	15 (6 I 9 II)
72% (31% I 54% II 15% III)	28% (40% I 60% II)
Total of F_4 , F_5 , F_6 :	
142 (44 I 57 II 41 III)	55 (22 I 28 II 5 III)
72% (31% I 40% II 29% III)	28% (40% I 51% II 9% III)

We see that the tumor incidence in the later generations is almost identical with the incidence of the earlier generations. However, the tumors appear now somewhat earlier than formerly.

In the earlier generations the record of tumor age was as follows:

I Period: 120 mice	9 tumors = 7%	} 27% {	} 1:11 + III = 1:6.6
II Period: 75 mice	14 tumors = 19%		
III Period: 36 mice	10 tumors = 27%		
			1:11 = 1:2.7
			II:III = 1:1.4
			1 + 11:III = 1:1

In the later generations the tumor age was as follows:

I Period: 197 mice	22 tumors = 11%	} 28%	}	1: II + III = 1:3
II Period: 131 mice	28 tumors = 21%			I: II = 1:2
III Period: 46 mice	5 tumors = 11%			II: III = 2:1
				1 + II: III = 3:1

The tumors belong now to the I age class, while formerly they belonged to the II age class.

The records of October, 1915, which were intermediate between those of the old and new groups, were as follows:

	<i>Without tumor</i>	<i>With tumor</i>
F ₃ F ₄ , F ₅	38 (13 I 13 II 12 III)	23 (10 I 12 II 1 III)
	62% (34% I 34% II 32% III)	38% (44% I 52% II 4% III)

The number of mice in this group is somewhat smaller and the tumor incidence is 38 per cent, slightly higher than in the other groups; but the percentage is on the whole of the same order as in the other records. This group, as well as the more recent groups, belongs to the first age class. The tumor rate of the *London* strain as a whole remains constant through the successive generations; this strain nevertheless is a composite of different substrains. Two substrains were detached from the main strain and each substrain was inbred and prevented from interbreeding with other substrains. These substrains differed in tumor rate from the main strain:

Substrains

(a) The offspring of tumor mouse 481, and (b) *London blue and white*, descendants of a group of *London* mice with blue and white color. (a) Family of 481 (offspring of an imported *blue London* mouse in which a tumor developed later.)

1. Records of October, 1915:

	<i>Without tumor</i>	<i>With tumor</i>
F ₂ and F ₃	18 (10 I 5 II 3 III)	0

2. More recent records:

F₃, F₄, F₅ 25 (8 I 5 II 12 III) 0

In both groups tumors are entirely lacking through the course of five generations.

(b) *London blue and white*:

F ₄ and F ₅	14 (10 I 3 II 1 III)	17 (8 I 7 II 2 III)
	45% (71% I 22% II 7% III)*	55% (47% I 41% II 12% III)

While in this case the tumor rate is higher, this may be accidental and due to the occurrence of a relatively large number of tumors in F₄. Here of 13 mice, 11 had tumors. In the fifth generation the tumor rate was again the one typical for *London* mice, viz., 33 per cent. The tumor age of this group was similar to the one of the other *London* mice; it stood between the first and second age class. The tumor rate of 481 is undoubtedly much lower than the average. It is, therefore, in confirmation of our previous results, possible to split off from larger groups, which were not quite homogeneous, substrains with a different tumor rate.

STRAIN LONDON + (EUROPEAN + 103) F₃

Previously we have considered mainly the F₁ and F₂ generations, with an admixture of a few groups of the F₃ and of one group of the F₄ generation. We have now added to these groups a few mice whose records were written up October, 1915.

The former records were as follows:

<i>Without tumor</i>	<i>With tumor</i>
80 (12 I 33 II 35 III)	5 (1 I 2 II 2 III)
95% (15% I 41% II 44% III)	5% (20% I 40% II 40% III)

The tumor age is somewhere between the II and III age class. The new records give mainly the data of the third and fourth generations with some additional data concerning the second and fifth generations.

F ₂	5 (2 I 1 II 2 III)	1 III
	83% (40% I 20% II 40% III)	7% (100% III)
F ₃	20 (3 I 7 II 10 III)	2 (1 II 1 III)
	91% (15% I 35% II 50% III)	9% (50% II 50% III)
F ₄	26 (8 I 7 II 11 III)	2 (1 II 1 III)
	93% (31% I 27% II 42% III)	7% (50% II 50% III)
F ₅	7 (1 I 6 III)	
	100% (14% I 86% III)	

Total of the later generations:

58 (14 I 15 II 29 III)	5 (2 II 3 III)
92% (24% I 26% II 50% III)	8% (40% II 60% III)

The tumor incidence is almost identical in both sets of records. In the individual generations no marked deviation occurs even when the number of mice used is not very great. The tumors appear somewhat later than in the former records; they belong to the fourth age class. However, with such a relatively small number of tumor mice in low tumor strains, a difference in the appearance of one single tumor causes a shifting into another age class. We must, therefore, disregard shifting of age classes in such cases and consider generally whether or not a strain belongs to the earlier or later age classes.

STRAIN HEITLER

In our former paper we gave the records of the first, second, and third generations. We also mentioned a small number of mice of the fourth generation. The records of October, 1915, concern the third, fourth, and fifth generations.

The former records were:

<i>Without tumor</i>	<i>With tumor</i>
74 (42 I 18 II 14 III)	28 (4 I 24 II)
73% (56% I 24% II 20% III)	27% (14% I 86% II)

The tumors belong to the second age class. The greater number in the second period is compensated for by the absence of tumors in the III Period.

The new records of October, 1915, are as follows:

<i>Without tumor</i>				<i>With tumor</i>			
73	(36 I	20 II	17 III)	21	(8 I	9 II	4 III)
77.7%	(49% I	27.4% II	23.6% III)	22.3%	(38% I	43% II	19% III)

The tumor rates in the earlier and later generations are very similar (27 per cent and 22.3 respectively); also the tumor age is not very different. The tumors stand somewhere between the first and second or third age class, but the tumors appear now somewhat earlier than previously.

$$1: II + III = 1: 4.4$$

$$II: III = 1: 1$$

$$1: II = 1: 2.1$$

$$1 + II: III = 11.4$$

STRAIN $S\frac{1}{2} + 328$ (ENGLISH SABLE)

The older records showed a tumor incidence of 61 per cent. The tumors belonged to the first age class. The records from October, 1915, have been added to the old ones; both combined are as follows:

<i>Without tumor</i>				<i>With tumor</i>			
F ₁	7	(4 I	3 II)	2	(1 I	1 II)	
F ₂	17	(12 I	1 II 4 III)	14	(7 I	6 II	1 III)
F ₃	12	(8 I	4 II)	32	(22 I	9 II	1 III)
F ₄	21	(16 I	5 II)	13	(10 I	3 II)	
Total:							
F ₁ , F ₄	57	(40 I	13 II 4 III)	61	(40 I	19 II	2 III)
	49%	(70% I	23% II 7% III)	51%	(65 $\frac{2}{3}$ % I	31% II	3 $\frac{1}{3}$ % III)

The newer records are as follows:

	<i>Without tumor</i>	<i>With tumor</i>
F ₃	35 (16 I 16 II 3 III) 47% (46% I 46% II 8% III)	39 (15 I 20 II 4 III) 53% (39% I 51% II 10% III)
F ₄	27 (20 I 4 II 3 III) 64% (74% I 15% II 11% III)	15 (10 I 4 II 1 III) 36% (67% I 26% II 7% III)
F ₅	22 (13 I 8 II 1 III) 33% (58% I 37% II 5% III)	44 (25 I 19 II) 64% (57% I 43% III)
F ₆	7 (3 I 4 II) 47% (43% I 57% II)	8 (3 I 5 II) 53% (37% I 63% II)
F ₇	2 (2 I) 50%	2 (2 I) 50%

We may add to this record the record of two special families of $8\frac{1}{2} + 328$, namely the offspring of tumor mouse 1113 and of tumor mouse 1075 which were $8\frac{1}{2} + 328$ F₃ July, 1914, groups.

Offspring of no. 1113.

	<i>Without tumor</i>	<i>With tumor</i>
F ₁ , F ₂ , F ₃	2 (2 I) 12% (100% I)	14 (13 I 1 II) 88% (93% I 7% II)

Offspring of no. 1075:

F ₁ , F ₂ , F ₃	4 (3 I 1 II) 40% (75% I 25% II)	6 (4 I 1 II) 60% (67% I 33% II)
--	------------------------------------	------------------------------------

If we combine the records of these special families with the newer records of Strain $8\frac{1}{2} + 328$ we obtain the following total

	<i>Without tumor</i>	<i>With tumor</i>
	99 (59 I 33 II 7 III) 43% (60% I 33% II 7% III)	128 (72 I 5 II 5 III) 57% (56% I 40% II 4% III)

We see, therefore, that in the earlier records (F₁-F₄) the tumor incidence is very similar to the later records (F₃-F₇), namely, 51 per cent as compared to 57 per cent.

We furthermore find that in special families within the larger strain the tumor rate was high and on the whole similar to that

of the main strain. Of course, whenever the number of observed mice becomes relatively small, the variations due to chance are increased.

In another substrain 782a of $(8\frac{1}{2} + 328)$ F₂, the records of which are not included in the main strain, the figures are as follows:

a. Former records:

F ₁ , F ₂	7 (5 I 2 II)	15 (8 I 7 II)
	32% (71.5% I 28.5% II)	68% (53.5% I 46.5% II)

b. Later records:

F ₂ , F ₃ , F ₄	21 (15 I 5 II 1 III)	33 (21 I 12 II)
	39% (71% I 24% II 5% III)	61% (64% I 36% II)

We see then (1) That the tumor incidence in the later generations is approximately the same as that in earlier generations; and (2) that special families or substrains have the same tumor incidence as the main strain.

The tumor age also remains the same in the later and in the earlier records in the main strain as well as in the individual families.

a. Old records (with the addition of the October, 1915, records):

I: II + III = 1:2.4	1: II = 1: 1.5
II: III = 1.5: 1	1 + II: III = 2.6: 1

b. New records:

1: II + III = 1: 3	1: II = 1: 1.7
II: III = 1.25: 1	1 + II: III = 2: 1

Both records are very similar; in both the tumors belong to the first age class, or appear even slightly earlier. The same holds good for the substrain 782a.

Here the old records are:

$$1: II = 1: 1.7$$

The new records are:

$$I:II = 1:1.5$$

In both records all the tumors appear in the first and second periods and the tumors belong to the first age class. Tumor age as well as tumor incidence remain in this case, therefore, constant in the course of successive generations and in substrains or special families.

Substrain 415

No. 415 is a substrain of 101 + (*European* + 103). No. 415 was a tumor mouse which belonged to the F_2 generation of the main strain. In the main strain the tumor incidence is intermediate between that of the parents, viz., 34 per cent. The tumors belong to the IV age class. In substrain 415 the tumor rate is distinctly lower; it corresponds to that of the mother.

The earlier generations had the following record:

	<i>Without tumor</i>	<i>With tumor</i>
F_1, F_2, F_3	16 (8 I 3 II 5 III)	3 (2 I 1 III)
	84% (50% I 19% II 31% III)	16% (67% I 33% III)

There is an intermediate record from October, 1915:

F_3	5 (4 I 1 III)	5 (1 I 4 II)
-------	---------------	--------------

The later records are as follows:

F_3	21 (6 I 8 II 7 III)	2 (1 II 1 III)
F_4	12 (5 I 6 II 1 III)	4 (1 I 3 II)
F_5	3 (1 I 2 II)	
F_6	18 (16 I 2 II)	1 (1 II)
Total:		
F_3, F_6	54 (28 I 18 II 8 III)	7 (1 I 5 II 1 III)
	89% (52% I 33% II 15% III)	11% (14½% I 71% II 14½% III)

As in the main strain the tumors do not appear early in life. The total of all the generations (F_1 - F_6) of 415 is as follows:

<i>Without tumor</i>	<i>With tumor</i>
70 (36 I 21 II 13 III)	10 (3 I 5 II 2 III)
87.5% (52% I 30% II 18% III)	12.5% (30% I 50% II 20% III)

The tumor age is as follows:

$$I: II + III = 1:7$$

$$II: III = 1:1.1$$

$$1: II = 1:3.2$$

$$1 + II: III = 1.2:1$$

The tumors belong to the II age class. They appear, therefore, somewhat earlier than in the main strain, which belonged to the fourth age class. We cannot attribute much importance to such differences in the tumor age, for it is liable to occur whenever the number of tumors is relatively small.

STRAIN 121 (ENGLISH + TAN) + CREAM

The former records included the F_1 - F_4 generations:

F_1 - F_4 53 (20 I 22 II 12 III)	39 (16 I 18 II 5 III)
58% (37% I 42% II 21% III)	42% (41% I 46% II 13% III)
II + III 59% III 69%	II + III 41% III 31%

This strain belonged to the II age class.

The intermediate records from October, 1915, are as follows:

F_3 and F_4 49 (23 I 15 II 11 III)	15 (4 I 6 II 5 III)
76½% (46½% I 31% II 22½% III)	23½% (27% I 39⅔% II 33⅓% III)

The tumor incidence is here lower than in the former records, but the rate is still intermediate between that of the two parents.

The same applies also to the new records:

F_3 9 (3 I 2 II 4 III)	3 (1 I 2 II)
75% (33⅓% I 22% II 45% III)	25% (33⅓% I 66⅔% II)
F_4 31 (5 I 17 II 9 III)	9 (3 I 4 II 2 III)
77½% (16% I 55% II 29% III)	22½% (33% I 44% II 23% III)

F_5	31 (5 I 13 II 13 III)	12 (6 I 4 II 2 III)
	72% (16% I 42% II 42% III)	28% (51% I 33% II 16% III)
F_6	10 (6 I 4 II)	4 (2 I 2 II)
	72% (60% I 40% II)	28% (50% I 50% II)
F_7	1 (1 II)	0
Total of the later generations:		
F_5-F_7	82 (19 I 37 II 26 III)	28 (12 I 12 II 4 III)
	74½% (23% I 45% II 32% III)	25½% (43% I 43% II 14% III)

The tumor incidence in the later generations is similar to that of the October records and lower than that of the earlier records.

Tumor age of the first generations; II age class:

$$\begin{array}{ll} 1: II + III = 1: 8.8 & 1: II = 1: 4.4 \\ II: III = 1: 1 & 1 + II: III = 1.2: 1 \end{array}$$

The tumors of the October, 1915, records stand somewhere between the II and III age class, but are nearer the II age class:

$$\begin{array}{ll} 1: II + III = 1: 8 & 1: II = 1: 2.8 \\ II: III = 1: 1.8 & 1 + II: III = 1: 1.35 \end{array}$$

Recent records:

$$\begin{array}{ll} 1: II + III = 1: 2.5 & 1: II = 1: 1.4 \\ II + III = 1.2: 1 & 1 + II: III = 2.1 \end{array}$$

The tumors belong now to the first age class; they appear relatively earlier, notwithstanding the decrease in the tumor rate. This is mainly due to the fact that in the later generations the non-tumor mice reach a higher age. Thus the number of tumor mice in the first age period is relatively increased. If we compare the proportion of tumor mice in the various age periods, we find in the later generations almost the same figures as in the earlier ones.

In the earlier generations:

$$41\% \text{ I } 46\% \text{ II } 13\% \text{ III}$$

In the later generations:

43% I 43% II 14% III

STRAIN ENGLISH, OCTOBER, 1915, RECORDS

Some groups consisting of *English Sable* and of some other high tumor rate English mice, F₄ and F₇:

<i>Without tumor</i>	<i>With tumor</i>
13 (9 I 3 II 1 III)	11 (7 I 3 II 1 III)
54% (70% I 23% II 7% III)	46% (64% I 27% II 9% III)

The tumor rate is still higher although slightly lower than the typical *English* tumor rate; however, the number of mice is relatively small and this might account for the slight variation. The tumors belong to the first age class as is characteristic of the strain *English*.

Substrain English Sable

Former records (F₁-F₆):

76 (49 I 19 II 8 III)	176 (115 I 48 II 13 III)
30% (65% I 25% II 10% III)	70% (66% I 27% II 7% III)

I age class:

I: II + III = 1:2.8	I: II = 1:1.5
II: III = 1:1	1 + II: III = 1.9:1

New records (F₄-F₇), with exception of family 437.

16 (9 I 4 II 3 III)	32 (13 I 17 II 2 III)
33% (57% I 25% II 18% III)	67% (41% I 53% II 6% III)

The tumor rate remains in the later generations almost the same as in the former ones. The age class remains also approximately the first.

$$I: II + III = 1:4$$

$$I: II = 1:2.4$$

$$II: III = 1.4: 1$$

$$1 + II: III = 2.3: 1$$

While the proportion of the tumors in I: II period is slightly lower, the proportion of the tumors in II: III age class is somewhat higher as compared to the corresponding figures in the earlier generations. The tumor age is slightly higher than formerly; however, the number of mice used is smaller. Among the *English Sable* one separate family, 437 (*English Sable* F₂), was observed which had a slightly higher tumor rate than the average. The former records of 437 with some additional records were as follows:

<i>Without tumor</i>	<i>With tumor</i>
F ₁ -F ₅ 5 (5 I)	23 (12 I 11 II)
18% (100% I)	82% (52% I 48% II)

The tumors belong to the first age class.

The new records of family 437 are as follows:

F ₄ -F ₇ 3 (3 I)	24 (20 I 4 II)
11% (100% I)	89% (84% I 16% II)

Here again the high tumor rate is sustained in the later generations and the tumors appear at an early age. We see, then, that within a certain strain a higher tumor rate of a selected family is maintained, and, furthermore, that the tumor age remains the same. In neither of the generations does a tumor appear in the III age period.

There were a few smaller families belonging to substrain *English A* (177a, 881a, 869a 281).

Their tumor rate was:

<i>Without tumor</i>	<i>With tumor</i>
17 (13 I 3 II 1 III)	19 (15 I 4 II)
44% (76% I 18% II 6% III)	56% (79% I 21% II)

The high tumor rate is maintained and the tumors belong as usual in this strain to the I age class.

Substrain English Silver

English Silver was a substrain of English that differed markedly from the other substrains; its tumor rate was very low.

The former records were as follows:

<i>Without tumor</i>	<i>With tumor</i>
109 (44 I 49 II 16 III)	8 (4 I 4 II)
93% (41% I 45% II 14% III)	7% (50% I 50% II)

Since then we have obtained a few more records which show the same low tumor rate:

9 (3 I 5 II 1 III)	1 (1 II)
90% (33% I 56% II 11% III)	10% (100% II)

The number of the additional mice is too small for the determination of the tumor age.

Substrain English Silver Fawn

We gave the records of two groups of this strain in our previous paper. It indicated a low tumor rate.

<i>Without tumor</i>	<i>With tumor</i>
13 (6 I 7 II)	0

The later records confirm the conclusion that this substrain differs markedly from the majority of English substrains and approaches the tumor rate of *Silver*, from which they were originally split off and from which they differed only slightly in color.

<i>Without tumor</i>	<i>With tumor</i>
37 (14 I 10 II 13 III)	7 (4 II 3 III)
84% (38% I 27% II 35% III)	16% (57% II 43% III)

The tumor rate is slightly higher than in *Silver*. The tumors appear here distinctly later than in the other English substrains and also later than they did in *English Silver*. Considering the relatively small number of tumor mice observed in the case of the recent groups of *English Silver* and *Silver Fawn*, a determination of the age class of these tumor mice can be made only with reservation.

STRAIN CREAM

This is a strain differing markedly from the English in tumor rate and tumor age. The tumor rate is low and the tumors appear late.

The former records of *Cream* were as follows:

<i>Without tumor</i>	<i>With tumor</i>
221 (63 I 91 II 67 III)	5 (2 II 3 III)
98% (29% I 41% II 30% III)	2% (40% II 60% III)
II + III 97% III 96%	II + III 3% III 4%

This record does not include certain substrains like *Cream X* and *Cream G*.

The new records of the later generations are as follows:

Cream A (mostly black color):

<i>Without tumor</i>	<i>With tumor</i>
105 (32 I 41 II 32 III)	9 (2 I 3 II 4 III)
92% (30.5% I 39% II 30.5% III)	8% (22% I 33% II 45% III)

The tumor rate is very low and the tumors appear late.

Cream B (mostly white color):

<i>Without tumor</i>	<i>With tumor</i>
72 (24 I 21 II 27 III)	17 (1 I 9 II 7 III)
87% (33% I 28% II 39% III)	19% (6% I 53% II 41% III)

There is included in this substrain a group with a somewhat higher tumor rate. These mice are the offspring of a family which was very prolific (June, 1914, family).

This group had the following tumor rate:

<i>Without tumor</i>	<i>With tumor</i>
26 (6 I 6 II 14 III)	14 (1 I 6 II 7 III)
65% (23% I 23% II 54% III)	35% (7% I 43% II 50% III)

While the tumor rate is here higher, the tumors appear late. The rest of this substrain, *Cream B*, with the exclusion of the June, 1914, family, has the typical low tumor rate.

<i>Without tumor</i>	<i>With tumor</i>
46 (18 I 15 II 13 III)	3 (3 II)
94% (39% I 33% II 28% III)	6% (100% II)

We may, therefore, conclude that no fargoing change has taken place in the *Cream* strain as a whole in regard to tumor incidence or tumor age, and that the change which affects apparently the whole strain is due to the prevalence within the strain of a certain family with a tumor rate that is higher than the average.

Substrain Cream Black (offspring of the original *Cream*, but mostly with black color)

a. Intermediate records of October, 1915.

<i>Without tumor</i>	<i>With tumor</i>
74 (26 I 27 II 21 III)	3 (1I 2 III)
96% (35.5% I 36.5% II 28% III)	4% (33 $\frac{1}{3}$ % I 66 $\frac{2}{3}$ % III)

b. The newer records of *Cream Black* are as follows:

<i>Without tumor</i>	<i>With tumor</i>
87 (31 I 25 II 31 III)	11 (3 I 2 II 6 III)
89% (35% I 30% II 35% III)	11% (27% I 19% II 54% III)

Although in the newer records of this substrain the tumor rate is still very low, it is somewhat higher than in the intermediate records and in *Cream* as a whole. The tumors appear late in life as usual in *Cream* strain.

Substrain Cream X

a. Old records:

<i>Without tumor</i>	<i>With tumor</i>
130 (48 I 45 II 37 III)	5 (2 I 3 II)
96% (37% I 35% II 28% III)	4% (40% I 60% II)

b. Intermediate records of October, 1915.

<i>Without tumor</i>	<i>With tumor</i>
75 (26 I 26 II 23 III)	2 (1 II 1 III)
97.4% (34.5% I 34.5% II 31% III)	2.6% (50% II 50% III)

c. New records:

<i>Without tumor</i>	<i>With tumor</i>
48 (13 I 20 II 15 III)	0
100% (27% I 42% II 31% III)	0%

The total of the new records (excluding records of October, 1915) of these groups of *Cream* including *Cream X* is as follows:

<i>Without tumor</i>	<i>With tumor</i>
312 (100 I 107 II 105 III)	37 (6 I 14 II 17 III)
89½% (32% I 35% II 33% III)	10½% (16% I 37% II 47% III)

While the tumor rate is still very low, it is somewhat higher than previously, and the tumors appear now at a slightly earlier period in life. The total of the new records of the corresponding substrains was as follows:

<i>Without tumor</i>	<i>With tumor</i>
98% (29% I 41% II 30% III)	2% (40% II 60% III)

There is one special family of *Black Cream* which was detached from the main strain for special reasons and called *Cream Y*. In this family the tumor rate was as follows:

<i>Without tumor</i>	<i>With tumor</i>
14 (6 I 5 II 3 III)	0
100% (43.5% I 35% II 21.5% III)	0%

Here tumors were absent.

Tumor age of the *Creams* is as follows:

a. The tumor age in the old *Cream* records (with exclusion of *Cream X* and *Y*.)

$$1: II + III = 1:00$$

$$1: II = 1:00$$

$$II: III = 1:3.6$$

$$1 + II: III = 1:3.6$$

The tumors appear later than in the IV age class.

In the new *Cream* records the tumor age is as follows:

b. Cream A:

$$1: II + III = 1:8.7$$

$$1: II = 1:2.1$$

$$II: III = 1:3$$

$$1 + II: III = 1:2$$

This corresponds to the IV age class as far as the relation of II: III, and to the II age class as far as the relation of the I: II period is concerned.

c. Cream B:

$$1: II + III = 1:32$$

$$1: II = 1:13$$

$$II: III = 1:1.5$$

$$1 + II: III = 1:1.4$$

This corresponds to the IV age class.

d. The offspring of the prolific group among Cream B:

$$1: II + III = 1:20$$

$$1: II = 1:7$$

$$II: III = 1:1.8$$

$$1 + II: III = 1:1.6$$

Notwithstanding the higher tumor rate in this group, the tumors belong likewise to the IV age class.

e. Cream Black:

$$1: II + III = 1:6.7$$

$$1: II = 1:1$$

$$II: III = 1:5.5$$

$$1 + II: III = 1:2.8$$

The relation of the I to the II period places these tumors in the II age class, but as far as the relation of the II: III period is concerned, they belong to the IV age class.

f. The former Cream X records:

$$1: II = 1:2.3$$

$$1: II + III$$

$$II: III = 3.50$$

$$1 + II: III = 5:0$$

The tumors belong to the I age class.

g. Total of all new Cream, including Cream X:

$$1: II + III = 1:7$$

$$I: II = 1:3.4$$

$$II: III = 1:1.2$$

$$1 + II: III = 1:1$$

The tumors belong to the II age class.

The tumors belong to the late age classes, and the record is maintained in later generations.

STRAIN NO. 8

The former figures for this strain are as follows:

	<i>Without tumor</i>	<i>With tumor</i>
F ₂ -F ₁₀	149 (70 I 33 II 37 III) 9 unknown age 70% (47% I 22% II 25% III) II + III 60% 111 63%	64 (17 I 24 II 23 III) 30% (29% I 37% II 34% III) II + III 38% III 37%

The intermediate October, 1915, and new records comprise F₁₂, F₁₃, F₁₄.

	<i>Without tumor</i>	<i>With tumor</i>
27 (6 I 9 II 12 III)		3 (3 III)
90% (22.5% I 35% II 44.5% III)		10% (100% III)

In this group there was a decrease in the tumor rate which became noticeable as early as the F₇ generation. If we divide the old records into two groups, the first group comprising F₂-F₆ and the second F₇-F₁₀, we find a marked difference.

	<i>Without tumor</i>	<i>With tumor</i>
I Group	96 (44 I 22 II 30 III)	49 (17 I 18 II 14 III)
F ₂ -F ₆	66% (46% I 23% II 31% III)	34% (35% I 38% II 28% III)
II Group	53 (29 I 14 II 10 III)	15 (6 II 9 III)
F ₇ -F ₁₀	78% (55% I 26% II 19% III)	22% (40% II 60% III)

We notice, then, in this strain, a gradual decrease in the tumor rate in the later generations.

With tumors: F₂-F₆ 34% F₇-F₁₀ 22% F₁₂-F₁₄ 10%

At the same time the tumors appear correspondingly later in life. The first generations belong to the II, the later ones to the IV age class. At first approximately one-third of the tumors appear in the first period, in the second group none appear in the first period, but four-tenths appear in the second period, while at last all the tumors appear in the III period of life. Parallel with this decrease in the tumor rate and the increase in tumor age, we find a decrease in the fertility of this strain.

STRAIN $8\frac{1}{2}$

In the former records (F_1 - F_5), with the addition of a few F_6 mice, the figures were as follows:

<i>Without tumor</i>	<i>With tumor</i>
131 (43 I 52 II 36 III)	27 (6 I 17 II 4 III)
83% (33% I 40% II 27% III)	17% (22% I 63% II 15% III)
II + III 81% III 90%	II + III 19% III 10%

Intermediate records from October, 1915:

<i>Without tumor</i>	<i>With tumor</i>
F_4 - F_6 28 (15 I 8 II 5 III)	0
100% (54% I 28% II 18% III)	0%

New records F_6 - F_8 :

<i>Without tumor</i>	<i>With tumor</i>
50 (20 I 25 II 5 III)	5 (1 I 3 II 1 III)
91% (40% I 50% II 10% III)	9% (20% I 60% II 20% III)

The tumor frequency was low from the beginning. A decrease took place in the later generations, somewhat as in strain no. 8. The tumors also appear now somewhat later than previously.

$$1: \text{II} + \text{III} = 1: 13$$

$$\text{II} + \text{III} = 1: 1.9$$

$$1: \text{II} = 1: 4.5$$

$$1 + \text{II}: \text{III} = 1: 1.5$$

In the later generations they stood between the III and IV age class, while formerly they belonged to the II age class.

STRAIN EUROPEAN

In our previous communications we distinguished two groups, the ordinary *European* and the descendants of the *Trio*. The figures for the sum of both were as follows:

<i>Without tumor</i>				<i>With tumor</i>			
F ₂ -F ₅	95	(37 I 33 II 25 III)		18	(7 I 8 II 3 III)		
84%	(39% I 34% II 27% III)			16%	(39% I 44% II 17% III)		

Intermediate records of October, 1915; we again give the combined figures, the ordinary *European* and the descendants of the *Trio*.

<i>Without tumor</i>				<i>With tumor</i>			
F ₄ , F ₅ , F ₆	79	(28 I 33 II 18 III)		3	(2 I 1 II)		
	96.4%	(35.5% I 42% II 22.5% III)		3.6%	(67% I 33% II)		

The new records are as follows:

<i>Without tumor</i>				<i>With tumor</i>			
F ₅ -F ₈	66	(24 I 22 II 20 III)		2	(2 III)		
	97%	(37% I 33% II 30% III)		3%	(100% III)		

If we combine the intermediate and the new records, we obtain the following figures:

<i>Without tumor</i>				<i>With tumor</i>			
145	(52 I 55 II 38 III)			5	(2 I 1 II 2 III)		
96.7%	(36% I 37% II 27% III)			3.3%	(40% I 20% II 40% III)		

We see, then, that while strain *European* does not essentially change its character, remaining poor in tumors, the tumor rate decreases from 16 per cent to 3.3 per cent. Formerly the tumors stood between the I and II age class as far as the relations between I and II age period are concerned, and they belonged to the I age class as through the relations between the II and III periods. Now the tumors belong partly to the I

age class (I:II age period) and partly to the III-IV age class (II:III age periods). In determining the age class, we must remember that if the number of tumors in a strain is very small, a few tumor mice may accidentally change the age class of the strain. This is liable to occur in the case of strains with a very low tumor rate.

HYBRIDS (EUROPEAN +102 OR 103)

Former tumor rate of *European* +102, 14 per cent (17 per cent). The tumors belonged to the IV age class.

European +103 had formerly a tumor rate of 10 per cent.

The tumor rate of the total of these hybrids (including some mixed families) is 21 per cent:

Without tumor	With tumor
116 (35 I 24 II 57 III)	30 (2 I 9II 19 III)
79.5% (30% I 21% II 49% III)	20.5% (7% I 30% II 63% III)

The October, 1915, records of *European* +102 were as follows:

Without tumor	With tumor
F ₅ 21 (18 I 3 II)	I (1 II)

The new records of *European* +102:

Without tumor	With tumor
F ₄ , F. 44 (19 I 15 II 10 III)	1 (1 III)
97.8% (43% I 34% II 23% III)	2.2% (100% III)

The new records of *European* +103:

Without tumor	With tumor
F ₁ -F ₃ 6 (1 I 1 II 4 III)	2 (1 II 1 III)
75% (16½% I 16½% II 67% III)	25% (50% II 50% III)

Total:

Without tumor	With tumor
71 (38 I 19 II 14 III)	4 (2 II 2 III)
94.7% (53% I 27% II 20% III)	5.3 (50% II 50% III)

As formerly, the tumor rate is very low in the newer records, but the tumor rate is lower now than it was formerly. Again the tumors belong to the IV age class.

HYBRIDS (EUROPEAN +8 F₆)

Former records:

	<i>Without tumor</i>	<i>With tumor</i>
F ₁ -F ₄	88 (31 I 19 II 38 III) 70% (35% I 22% II 43% III)	37 (8 I 20 II 9 III) 30% (22% I 54% II 24% III)

Combined records of October, 1915, and new records:

	<i>Without tumor</i>	<i>With tumor</i>
F ₃ -F ₆	20 (7 I 5 II 8 III) 77% (35% I 25% II 40% III)	6 (1 I 2 II 3 III) 23% (17% I 33% II 50% III)

The tumors belonged formerly to the II age class; they now stand between the II and III age class. The new records are therefore very similar to the old ones. The tumor rate is now slightly lower and the tumors appear only a little later than formerly.

HYBRIDS (EUROPEAN +102) F₁+8½ F₄

Former records:

	<i>Without tumor</i>	<i>With tumor</i>
F ₁ -F ₆	396 (101 I 112 II 183 III) 84% (25% I 28% II 47% III)	77 (8 I 19 II 50 III) 16% (10% I 25% II 65% III)

The tumors belong to the IV age class.

Records of October, 1915.

F ₂ -F ₅	59 (17 I 15 II 27 III) 87% (29% I 25% II 46% III)	9 (2 I 4 II 3 III) 13% (22% I 44⅔% II 33⅓% III)
--------------------------------	---	---

The tumors stand between the II and III age classes.

New records:

	<i>Without tumor</i>	<i>With tumor</i>
F_3-F_7	50 (15 I 12 II 23 III) 82% (30% I 24% II 46% III)	11 (1 I 3 II 7 III) 18% (9% I 27% II 64% III)

The tumor rate is approximately unchanged.

The tumor age of the new records also is similar to the former records:

$$1: \text{II} + \text{III} = 1: 19$$

$$\text{II}: \text{III} = 1: 3.3$$

$$1: \text{II} = 1: 5$$

$$\text{I} + \text{II}: \text{III} = 1: 2.4$$

The tumors belong again to the IV age class.

HYBRIDS (EUROPEAN 151 + II DAUGHTER OF NO. 10 (NOVEMBER
8TH STRAIN)

Former records:

F_1-F_4	34 (15 I 16 II 3 III) 35% (44% I 47% II 9% III) II + III 31%	62 (20 I 33 II 9 III) 65% (32% I 54% II 14% III) II + III 69% II Age class
-----------	---	---

Records of October, 1915 and new records combined (including the records of family 593):

F_4-F_7	51 (32 I 12 II 7 III) 56% (63% I 23% II 14% III) II + III 45%	40 (17 I 15 II 8 III) 44% (43% I 37% II 20% III) II + III 55%
-----------	--	--

The tumor rate of this strain remains high; it is, however, not quite so high as before. This is in part due to the great number of deaths in the first age class among the non-tumor animals in the new records. In these "II+III" shows, therefore, a somewhat higher tumor rate. The age class of this tumor

is approximately the II class. It belonged formerly, also, to the II age class.

$$\begin{array}{ll} 1: \text{II} + \text{III} = 1:5 & 1: \text{II} = 1:2 \\ \text{II}: \text{III} = 1:1.5 & 1 + \text{II}: \text{III} = 1:1.2 \end{array}$$

HYBRIDS ($8\frac{1}{2}$ + II DAUGHTER OF NO. 10)

Former records:

	<i>Without tumor</i>	<i>With tumor</i>
F ₁ -F ₄	42 (8 I 14 II 20 III) 51% (19% I 33% II 48% III)	40 (6 I 19 II 15 III) 49% (15% I 47½% II 37½% III)
II + III	50%	II + III 50%

New records:

	<i>Without tumor</i>	<i>With tumor</i>
F ₄ -F ₆	24 (8 I 9 II 7 III) 86% (33% I 38% II 29% III)	4 (4 II) 14% (100% II)
II + III	80%	II + III 20%

There is a noticeable decrease in the tumor rate which is probably not an accident due to the small number of mice in the new series. As before the tumors appear late.

STRAIN (EUROPEAN +103) F₁+III DAUGHTER OF NO. 10

Former records:

	<i>Without tumor</i>	<i>With tumor</i>
F ₁ -F ₃	140 (50 I 37 II 53 III) 83% (36% I 27% II 37% III)	28 (3 I 11 II 14 III) 17% (11% I 39% II 50% III)

These tumors belong to the IV age class.

Records of October, 1915:

	<i>Without tumor</i>	<i>With tumor</i>
F ₃ , F ₄	26 (13 I 13 II) 87% (50% I 50% II)	4 (2 II 2 III) 13% (50% II 50% III)

Here again the tumors belong to the IV age class.

New records:

	<i>Without tumor</i>	<i>With tumor</i>
F ₃ , F ₄ , F ₅	21 (7 I 7 II 7 III)	0
	100% (33 $\frac{1}{3}$ % I 33 $\frac{1}{3}$ % II 33 $\frac{1}{3}$ % III)	0%

The tumor rate remains throughout low, and tumors appear late, but a decrease apparently takes place in the later generations.

STRAIN (EUROPEAN (151) + I DAUGHTER OF NO. 10 (NOVEMBER 3RD) + 101 ENGLISH)

Former records:

	<i>Without tumor</i>	<i>With tumor</i>
F ₁ -F ₄	12 (8 I 2 II 2 III)	15 (10 I 5 II)
	45% (66% I 17% II 17% III)	55% (66 $\frac{2}{3}$ % I 33 $\frac{1}{3}$ % II) I Age class.

October, 1915, records:

F ₃ -F ₄	14 (9 I 5 II)	8 (3 I 3 II 2 III)
	64% (64% I 36% II)	36% (37.5% I 34.5% II 25% III)

New records:

	<i>Without tumor</i>	<i>With tumor</i>
F ₄ -F ₆	10 (7 I 2 II 1 III)	3 (3 I)
	77% (70% I 20% II 10% III)	23% (100% I)

Age of death of non-tumor mice, intermediate and new records combined:

$$24 (16 \text{ I } 7 \text{ II } 1 \text{ III}) = 66.6\% \text{ I } 29.2\% \text{ II } 4.2\% \text{ III}$$

While the number of mice in the new records is not large, still it is probable that an actual decrease in the tumor rate has taken place in this strain.

Tumor age of intermediate and new groups combined:

$$\begin{array}{ll} 1: \text{II} + \text{III} = 1:5 & 1: \text{II} = 1:1.4 \\ \text{II}: \text{III} = 1:3 & 1 + \text{II}: \text{III} = 1.1.7 \end{array}$$

They stand between I and II age classes in part, and in part they belong to III age class.

HYBRIDS (EUROPEAN + ENGLISH TAN (DAUGHTER OF TUMOR MOUSE 146)).

Former records:

	<i>Without tumor</i>	<i>With tumor</i>
F ₁ -F ₃	53 (13 I 25 II 17 III) 72% (24% I 43% II 33% III)	21 (3 I 13 II 5 III) 28% (14% I 62% II 24% III)
	II age class.	

In this case F₃ had a very low tumor rate.

F₁-F₂ gave a higher record:

23 (5 I 12 II 6 III) 56% (22% I 52% II 26% III)	18 (3 I 11 II 4 III) 44% (16% I 61% II 23% III)
--	---

The new records:

F ₄ -F ₆	19 (3 I 10 II 6 III) 58% (16% I 52% II 32% III)	14 (1 I 11 II 2 III) 42% (7% I 79% II 14% III)
--------------------------------	---	---

The new records are very similar to the F₁ and F₂ generations of the former records. But the F₃ generation of the former record had a very low tumor incidence.

Tumor age:

Formerly in I period	4%	now in I period	3%
Formerly in II period	22%	now in II period	38%
Formerly in III period	23%	now in III period	25%
= II class.			

= II class.

I: II + III = 1:21

I: II = 1:13

II: III = 1.5:1

I + II: III = 1.6:1

I: II = IV age class

II: III = I age class.

The large majority of the tumor mice die in this strain in the II age period.

HYBRIDS (SILVER + 10)

Former records:

<i>Without tumor</i>	<i>With tumor</i>
150 (54 I 50 II 46 III)	84 (26 I 29 II 29 III)
64% (35½% I 33½% II 31% III)	36% (32% I 34% II 34% III)
	II age class.

New records:

<i>Without tumor</i>	<i>With tumor</i>
4 (2 I 2 II)	7 (3 I 2 II 2 III)
36% (50% I 50% II)	64% (43% I 28.5% II 28.5% III)

While the number of mice is too small to allow an exact comparison with the tumor rate of the former records, still the record is of value as indicating that the low tumor rate of the *Silver* was not dominant over the *no. 10* strain and that the tumor rate was maintained fairly high.

HYBRIDS (CREAM + I DAUGHTER OF NO. 10)

Former records:

F ₁ -F ₄	112 (18 I 33 II 61 III)	62 (4 I 23 II 35 III)
	64% (16% I 29% II 55% III)	36% (6% I 37% II 57% III)
		IV age class.

New records:

F_4-F_6	35 (10 I 11 II 14 III)	12 (4 I 6 II 2 III)
	74% (28% I 31% II 41% III)	26% (33% I 50% II 17% III)

Age class:

I period:	8.5%	1: II + III = 1:3.7	1: II = 1:2.2
II period:	19%	II: III = 1.5:1	1 + II: III = 2.2:1
III period:	12.5%		

The tumor belongs to the I age class.

While the tumor rate is somewhat lower than previously, no essential change has taken place in this respect. The tumors appear earlier than previously; however, considering the relatively small number of tumors observed in the later generations no great importance can be attached to this change.

HYBRIDS (MICHIGAN WILD + ENGLISH 101)

Former records:

F_1-F_4	21 (14 I 4 II 3 III)	29 (13 I 9 II 7 III)
	42% (67% I 19% II 14% III)	58% (45% I 31% II 24% III)

I age class

New records (including October, 1915, records):

F ₃ , F ₅ , F ₆	15 (8 I 4 II 3 III)	5 (3 I 1 II 1 III)
	75% (53% I 27% II 20% III)	25% (60% I 20% II 20% III)
I Period:	15%	1: II + III = 1: 2.4 1: II = 1.3: 1
II Period:	11%	II: III = 1: 2.3 I + II: III = 1: 1
III Period:	25%	

The tumor age is partly I class, partly II and III classes. Here again a decrease in the tumor rate is noticeable. The tumor age remains essentially unchanged.

HYBRIDS (ENGLISH TAN AND GERMAN)

Former records:

<i>Without tumor</i>	<i>With tumor</i>
25 (11 I 7 II 2 III)	19 (10 I 7 II 2 III)
51% (55% I 35% II 10% III)	49% (52% I 36% II 12% III)
I age class	

New records:

<i>Without tumor</i>	<i>With tumor</i>
F ₃ -F ₇ 12 (4 I 6 II 2 III)	9 (4 I 5 II)
57% (33% I 50% II 17% III)	43% (44½% I 55½% II)
I age class	

The new records are very similar to the old ones. We cannot be certain about the character of this strain but we had reason to believe that it belongs to the class of English mice. The records bear out this suggestion, inasmuch as the tumor rate was high and the tumors appeared early, just as in the case of the *English* strain.

Former records:

<i>Without tumor</i>	<i>With tumor</i>
F ₁ -F ₅ 7 (3 I 3 II 1 III)	15 (6 I 8 II 1 III)

New records:

F ₄ -F ₆ 2 (2 II)	8 (8 I)
---	---------

While the number of mice observed is not great, the new records confirm the older ones.

Total:

<i>Without tumor</i>	<i>With tumor</i>
9 (3 I 5 II 1 III)	23 (14 I 8 II 1 III)
28% (33% I 56% II 11% III)	72% (60½% I 35% II 4½% III)

I Period: 44%	1: II: III = 1: 2.3	1: II = 1: 1.2
II Period: 53%	II: III = 1: 1	1 + II: III = 2:1
III Period: 50%		

I age class

STRAIN GERMAN

At no time did we have a large number of mice in this strain; however, all indications point to the conclusion that the tumor rate in this strain is fairly high.

Former records:

<i>Without tumor</i>	<i>With tumor</i>
10 (6 I 3 II 1 III)	10 (3 I 5 II 2 III)
50% (60% I 30% II 10% III)	50% (30% I 50% II 20% III)

II age class

Records of October, 1915:

<i>Without tumor</i>	<i>With tumor</i>
F ₄ -F ₆ 11 (7 I 4 II)	5 (3 I 2 II)
69% (64% I 36% II)	31% (60% I 40% II)

New records:

F ₇ 4 (2 I 2 II)	2 (2 I)
66⅔% (50% I 50% II)	33⅓% (100% I)

The tumor rate is somewhat lower than it was previously. The tumors appear early. Formerly the tumors belonged to the II age class. The small number of tumor mice in the later records makes the determination of the age class uncertain.

HYBRIDS (S + GERMAN)

Former records:

<i>Without tumor</i>	<i>With tumor</i>
F ₂ -F ₅ 144 (40 I 46 II 58 III)	100 (21 I 51 II 28 III)
	41% (21% I 51% II 28% III)

(Including a few mice from later generation.)

59% (28% I 32% II 40% III) II + III 41%
II + III 59% II age class

Records of October, 1915:

F₃, F₄, F₆ 27 (1 I 21 II 5 III) 8 (5 I 3 II)
77% (3.7% I 77.8% II 23% (62.3% I 37.5% II)
18.5% III)

New records:

F₄-F₉ 75 (22 I 24 II 29 III) 19 (4 I 12 II 3 III)
80% (30% I 32% II 20% (21% I 63% II 16%
38% III) III)

There is in this strain a noteworthy fall in the tumor rate, which was already apparent in the intermediate and was maintained in the new records:

New records:

I Period: 4.2% 1: II + III = 1:6.5 1: II = 1:4.3
II Period: 18% II : III = 2:1 1 + II: III = 2.5:1
III Period: 9%

The tumors stand between I and II age class, but are nearer the II age class.

Included among the *8 + German* in our previous report appeared a family which was the offspring of tumor mouse no. 240. This family we used for further hybridization and we therefore give this record:

NO. 240 F-F₃ = 8 + GERMAN F₄-F₇

Without tumor

With tumor

8 (4 I 3 II 1 III) 6 (4 I 2 II)
57% (50% I 37½% II 12½% III) 43% (67% I 33% II)

The tumor rate is here very similar to that of *8 + German*.

198 (ENGLISH) + (8+GERMAN) F₄

Former records:

<i>Without tumor</i>	<i>With tumor</i>
30 (22 I 7 II 1 III)	52 (27 I 25 II)
37% (74% I 23% II 3% III)	63% (52% I 48% II)
II + III 24%	II + III 76%

I age class

Records of October, 1915:

<i>Without tumor</i>	<i>With tumor</i>
F ₂ , F ₃ 8 (3 I 5 II)	20 (11 I 8 II 1 III)
29% (37% I 63% II)	71% (55% I 40% II 5% III)
1: II + III = 1:4	1: II = 1:1.4
II: III = 1:1.75	1 + II: III = 1:1

Between I and II age class

New records:

<i>Without tumor</i>	<i>With tumor</i>
F ₂ -F ₆ 61 (42 I 16 II 3 III)	46 (23 I 19 II 4 III)
57% (69% I 26% II 5% III)	43% (50% I 41% II 9% III)
II + III 45%	II + III 55%
I Period: 22.5%	1: II + III = 1:4.5
II Period: 45%	1: II = 1:2
III Period: 57%	II: III = 1:1.3
	1 + II: III = 1.2:1.

Between I and II age class

The tumor rate remains high, and higher in II + III than in the sum of all age classes, just as in the earlier records. On the whole, however, a decrease has taken place in the tumor rate from 63% to 43%. The tumor age has increased only slightly.

HYBRIDS (GERMAN + CARTER)

Former records:

<i>Without tumor</i>	<i>With tumor</i>
F ₁ -F ₄ 326 (79 I 88 II 159 III)	32 (4 I 11 II 17 III)
91% (24% I 27% II 49% III)	9% (12½% I 39% II 48½% III)

IV age class

I Period: 1%	1: II + III = 1:14	1: II = 1:4
II Period: 4%	II: III = 1:2.5	1 + II: III = 1:2
III Period: 10%		

New records:

<i>Without tumor</i>			<i>With tumor</i>		
F ₄ -F ₆	75 (32 I 22 II 21 III)		38 (16 I 13 II 9 III)		
	66½% (43% I 29% II 28% III)		33½% (43% I 34% II 23% III)		
I Period: 14%	1: II + III = 1:3.5		1: II = 1:1.4		
II Period: 20%	II: III = 1:1.5		1 + II: III = 1:1		
III Period: 30%					

The records of this strain had in all probability been abnormal, inasmuch as the tumor rate of the hybrids was much lower than that of either parent. Now the tumor rate has increased and has become similar to that of *Carter*. At the same time the tumors appear earlier.

One family of strain *German* + *Carter*, the offspring of tumor mouse No. 794 (= *German* + *Carter* F₃), was kept separate.

The records of No. 794 are as follows:

<i>Without tumor</i>			<i>With tumor</i>		
794 F ₁ -F ₄ = GERMAN + CARTER F ₄ -F ₇					
16 (8 I 5 II 3 III)			9 (1 I 4 II 4 III)		
64% (50% I 31% II 19% III)			36% (11% I 44½% II 44½% III)		
I Period: 4%	1: II + III = 1:20.5		1: II = 1:6		
II Period: 24%	II: III = 1:2.3		1 + II: III = 1:1.9		
III Period: 57%					

Tumors appear here late as they did in the early *German* + *Carter* records. They belong almost to the IV age class.

DISCUSSION

From an analysis of our records, we may conclude that a considerable majority of our tumor strains, viz., twenty strains or substrains, maintained a tumor rate approximately unchanged throughout the period of observation. On table 1 these strains are recorded. On the whole, the constancy of the percentages is surprising in most of the cases. In a few strains there is noticeable a very slight rise, in others a slight decrease in the tumor rate; the latter preponderates over the rise. In one case (*Cream + 10*) the descent is somewhat more marked (from 36 per cent to 26 per cent), so that it might be queried whether this strain would not, perhaps, have been more suitably placed among those showing a decline in the tumor rate. The strains in which the tumor rate is approximately constant comprise all kinds—high, as well as medium and low tumor rate strains.

There are only two strains in which the tumor rate has risen (table 2). Such an increase was found in some of the *Cream* substrains and in the *German* and *Carter* strain. While *Cream X* remained unchanged, on the whole, or fell slightly, we find a small increase in the case of *Cream A*; and among the *Cream* mice a substrain, *Cream B*, was developed, which had a noticeably higher tumor rate. The rate of *Cream B* rose to 19 per cent, while the average of *Cream* as a whole had been below 10 per cent. If we analyze still further the increase in the tumor rate of *Cream B*, we find that it affects principally one family (June, 1914, family); here the rate rose as high as 35 per cent, while in the rest of *Cream B* it remained typically low, namely, 6 per cent. We may, therefore, conclude that the rise in the tumor rate is due to the fact that certain families, which had from the beginning a potentially higher rate, but were at first not numerically important, gained in the course of time a certain numerical preponderance over the other substrains and thus caused apparently an increase in the tumor rate of the whole strain. This is an occurrence comparable to an apparent increase in the number of takes in transplanted tumors, which we have observed in certain strains in the case of continued trans-

TABLE 1
Unchanged or slightly decreasing tumor rate

TUMOR RATE			STRAIN	TUMOR AGE		
Previous	Inter- mediate	Recent		Previous	Inter- mediate	Recent
per cent	per cent	per cent				
27	38	28	<i>London</i> <i>481 London</i> <i>London blue white</i> <i>London + (European + 103) F₃</i> <i>Heitler</i>	II	I	I
5		55		II-III		I-II
27	22.3	8		III-I	I-II	IV
51		57 (Total)	$8\frac{1}{2} + 328$	I		I
		88	$1113 = (8\frac{1}{2} + 328)$ $1075 = (8\frac{1}{2} + 328)$ $782a = (8\frac{1}{2} + 328) F_2$ $415 = 101 + (\text{Europe} + 103)$	I Between I and III		I
68		60				II
16		61				
		11				
12.5			Total 415 ($F_1 - F_6$)	II		
34			<i>Main strain 101 + (Europe + 103)</i> <i>English</i> <i>English Sable</i> <i>437 (English Sable)</i> <i>English A</i> <i>English Silver</i> <i>Silver Fawn</i> <i>Cream X</i> <i>Cream Y</i>	IV		
		46				I
70		67		I		1 (II)
82		89		I		I
63		56	<i>European + 8F₆</i> $(\text{European} + 102) + 8\frac{1}{2} F_4$ <i>European + English Tan (146)</i> <i>Cream + 10</i> <i>English Tan + German</i> Uncertain	I		I
7		10		I		
Very low		16				IV
4	2.6	0		I		IV
0				IV		
30	23			II		II-III
16	13	18		IV	II-III	IV
$F_1 - F_2, F_1 - F_3$	28	42		II		IV-I
44						
36		26		IV		I
49		43		I		I
High		High		I		
8+ Ger- man		43	<i>No. 240 = 8 + German</i>	II		I
41						

plantation (2). In this case we could show that an apparent change in the susceptibility to tumor inoculation of a certain strain was only apparent, being due to the gradual numerical preponderance of a certain family within the strain. A certain family propagates more rapidly, either as a result of accident, or in consequence of a greater resistance to certain diseases, or as a result of a naturally greater prolificness. Another undoubted rise in the tumor rate was observed in the case of *Ger-*

TABLE 2
Increasing tumor rate

TUMOR RATE			STRAIN	TUMOR AGE		
Previous	Inter- mediate	Recent		Previous	Inter- mediate	Recent
<i>per cent</i>	<i>per cent</i>	<i>per cent</i>				
2 (Total)		8	<i>Cream A</i>	IV* (Total)		II-IV
		19	<i>Cream B</i> total			IV
		35	<i>Cream B</i> June 1914 family			IV
		6	<i>Cream B</i> without June 1914 family			
	4	11	<i>Cream Black</i>			II-IV
2		10½	Total <i>Creams</i> including <i>Cream X</i>	IV		II
36		64 ? small number	<i>Silver + 10</i>	II		
9		33½	<i>German + Carter</i>	IV		Almost I
		36	<i>No. 794-German + Car- ter</i>			Almost IV

*Total with exclusion of *Cream X* and *Y*.

man + Carter; where formerly the tumor rate had been but 9 per cent, the new records show a rate of 33 to 36 per cent. The former tumor rate in this case had been abnormally low, lower than that of either of the parents, but in the later records it approaches that of the mother strain. We should, therefore, have to explain the former low rate, rather than the later rise. This we are not able to do at present, but we suspect that here again the preponderance in earlier generations of a certain family with a low tumor rate was responsible for the very low

rate of the whole strain. The rise in *Silver + 10* may be without significance, considering the very small number of animals which composed the later generations.

Very much more considerable is the number of strains in which the tumor rate has decreased in the later generations (table 3). Thirteen strains show this fall, though quantitatively the decrease varies considerably in different strains. In some it is not very pronounced; thus in strain $S\frac{1}{2}$ there is a decrease from 17 per cent to 9 per cent only. In others, on the contrary, it is very distinct, as in $S\frac{1}{2} + II$ daughter no. 10, where the rate decreased from 49 per cent to 14 per cent, or in *Michigan Wild + English 101*, where it dropped from 58 per cent to 25 per cent. We shall now briefly consider the various strains in which a decrease took place.

1. *Strain 121 + Cream*. This strain was composed of mice of various colors—black, sable, agouti, blackeyed and pinkeyed yellow, smoky yellow, and lavender and silver with pink eyes. Groups composed of yellow breeders had few young, while those with dark colors had a larger number. They were medium-sized and moderately tame mice. The decrease, which began in early generations and continued throughout the period of our observations, is probably due to a selection within the whole strains of certain families with a lower rate.

2. *Strain 8*. This is one of the earliest strains, and is now inbred to the 17th generation. As a result, probably, of the long inbreeding, a change has gradually taken place; while at first it was relatively prolific (although not so fertile by far as some of the more recent strains), the prolificness has decreased in the course of inbreeding and, furthermore, the individual mice now grow more slowly than in the early generations. We may in all probability attribute the fall in tumor rate to the same factors that caused the decline in prolificness and growth rate. These changes are evidently due to the effects of inbreeding.

3. *Strain $S\frac{1}{2}$* . In this strain a decrease seems to have taken place from the F_4 generation on. In *European*, a fall began approximately when the fourth or fifth generation had been reached and continued in the following generations. This

TABLE 3
Decreasing tumor rate

TUMOR RATE			STRAIN	TUMOR AGE		
Previous	Inter- mediate	Recent		Previous	Intermediate	Recent
<i>per cent</i>	<i>per cent</i>	<i>per cent</i>				
42	23½	25½	121 + Cream	II	II-III	I
30	F ₁₂ -F ₆₄ 10		No. 8	F ₂ -F ₆ ; ½ in I Period	F ₇ -F ₁₀ ; one in I period; 4/10 in II period	F ₁₂ -F ₁₄ ; only in III period
F ₂ -F ₆ , 34	0	9	No. 8½	II		III-IV
F ₇ -F ₁₀ , 22	3.6	3	European	I-II and I		I and III-IV
17		5.6	European + 102 (103)	IV		IV
16						
21						
65 (69)	44 (55)		European 151 + II daughter of no. 10 (Nov. 8 strain)	II	I + II approximately	
49 (50)		14 (20)	8½ + II daughter of no. 10			
17	13	0.	(European + 103) F ₁ + III daughter of no. 1)	III		Late
55	36	23	(European 151 + I daughter of no. 10.) (November 3) + 101	IV	IV	IV
58		25	Michigan Wild + English 101	I	I-II-III	
50	31	33½	German	I-II (III)	I-II (III)	
41	23	20	8 + German	II		I-II near II
63	F ₂ -F ₃ 71	F ₇ -F ₅ 43	198 (English) + (8 + German) F ₄	II		age class
				I	I-II	I-II

strain has always been very prolific and began breeding at an exceptionally early age, especially for several generations after the mice had been imported; and even now, in the tenth generation, its members are maturing earlier than those of some other strains. Originally they were very unhealthy, being liable to typhus and to a disease affecting very young mice, or losing their young apparently from malnutrition without any apparent disease. Owing to these conditions they finally became extinct, with the exception of the descendants of one trio, which freed themselves of typhus and in time grew to be a most vigorous strain. The *European* were very small mice when imported and for the next few generations, but finally reached medium size. They are still prolific, and not now especially subject to any disease. We see in the behavior of this strain, in so far as the incidence of spontaneous tumors is concerned, a parallel to its behavior towards propagable neoplasms; only the change was in an inverse order. At a time when their susceptibility to our transplantable mouse tumor IX increased, the rate of incidence of spontaneous growths among them decreased; and we are probably justified in attributing those changes to the same cause, viz., to an alteration in the constitution of the strain. Owing to the ravages of disease, and especially of typhus, the main strain became almost extinct and all the later European mice were the offspring of a selected family, the *Trio*. These mice were more resistant to infection, and of somewhat larger size than the other European mice; and while they were still very prolific, they apparently reached maturity not quite so early as the mixed Europeans of the earlier generations. The change in the tumor rate goes parallel, therefore, to other changes in the character of this strain, both depending in all probability on the selection of a family differing somewhat in character from the main strain.

4. Among those strains that showed a decrease in tumor rate are four hybrid strains, where *European* entered in the composition of the strain. Two of these belonged to high tumor strains, while the other two were low. Usually the decline in the tumor rate began approximately in the F_4 generation. In about the

same generation a decrease took place in the tumor rate of *Michigan Wild* + 101, in *German*, and in 8 + *German*. In 198 + (8 + *German*) F_4 , the fall occurred even somewhat earlier. It is impossible for us at present to indicate with any degree of probability the cause of the decrease in tumor rate in these strains.

If we now inquire into the age at which tumor mice die in the various strains, we find on the whole that the earlier and later generations preserve their characteristics, although some changes do occur. In those strains where the tumor rate remained approximately constant (table 1), the following belonged formerly to the first age class: $8\frac{1}{2}$ + 328, with a substrain 782a and the various English substrains. In all these the later generations continue in the first age class. In regard to *English Silver*, the number of tumor mice is too small to allow a definite conclusion. It is, however, of interest that a substrain of English with very low tumor rate, *Silver fawn*, belongs to the fourth age class. This confirms our former conclusions that tumors tend, on the whole, to appear at a later period in life in strains where the tumor rate is low. As for (*European* + 102) + $8\frac{1}{2}$ F_4 , the strain belonged formerly to the IV age class, and the same holds good of the recent generations. *Cream X* now approaches the other *Cream* strains in their age class, which previously had been abnormally low. In the majority of the remaining strains, the age class has been somewhat intermediate between the first and fourth classes, and this condition is on the whole maintained, with the exception of *Cream* + 10, in which the tumors appear in later generations considerably earlier than formerly. However, in this case the number of tumor mice in our recent records is not very great. Still smaller was the number of mice observed in the substrain 240, and a change from the second to the first age class has, therefore, very little significance in this case.

If we now consider those strains in which the tumor rate increased (table 2), we find that in the *Cream* strains the high age class remains unchanged on the whole. And even those creams in which the tumor rate rose considerably still belong to the fourth age class. However, the creams as a whole have

now to be placed in the second class. In the main strain of *German + Carter*, simultaneously with an increase in the rate the tumors now appear earlier. In one substrain, however, the tumors continue to appear late, notwithstanding the increased tumor rate.

In those strains which show a decrease in the tumor rate, we find in the majority of cases an age class essentially unchanged, viz., in *European*, *European + 102* (103), *European 151 + II daughter of no. 10*, $8\frac{1}{2} + II$ daughter of no. 10, (*European + 103*) $F_1 + III$ daughter of no. 10, *Michigan wild + English 101*, $8 + German$, and $198 + (8 + German) F_4$. In others the age class rose in accordance with the decrease in tumor rate. This is especially noticeable in strain 8 where about one-third of the tumors in the earlier generation appeared during the first period, while in the intermediate generations none appeared in the last period of life. A similar rise in the age class is to be noted in strains $8\frac{1}{2}$ and (*European 151 + I daughter of no. 10*) + 101.

We may then conclude, on the whole, that the age class remains unchanged in the large majority of strains, and that the relation between tumor rate and age class (in the sense that a high rate is associated with the early appearance of tumors, and allow rate with their late appearance) becomes still more marked in after generations as a result of changes in the tumor age occurring in some cases. We must furthermore conclude that the age class depends upon a number of variables, especially upon the proportion of mice dying without tumors in the various age periods, and that this factor may produce certain variations in the age class in earlier and later generations of the same strains. Whenever the number of tumor mice in a certain strain is small, slight variations in the proportion of tumor mice dying in the different age periods may cause a considerable difference in the age class in which this strain is to be placed. We must not, therefore, attach too much importance to certain variations in the age class in strains where the tumor rate is low and the number of tumor mice small; it is, therefore, doubtful whether, for instance, the difference in the age class between *Silver + 10*

and *Cream* + 10, which we noticed in the earlier generations, is of more than accidental significance.

SUMMARY

1. Continued study of strains of mice in which we have established the tumor rate for earlier generations shows that in the majority of cases the rate remains the same throughout later generations. In most of these strains the constancy in the tumor rate is striking. In a few exceptional cases the rate has increased, but in a considerable number of strains there is a distinct fall.

2. If we inquire into the causes of these changes we may attribute them with great probability in certain cases to one of the following two factors: (a) to differences in resistance to disease, or in prolificness, in various families of the same strain, or to other more or less accidental factors, causing certain families with a different cancer rate to preponderate to an unequal degree in earlier and later generations; thus, an apparent change in the whole strain is due only to the effect of selection among certain constituents of the strain. (b) As the result of long continued inbreeding, certain characteristics of a strain change. The strain becomes less prolific and less vigorous, and hand in hand with this change goes a lowering of the tumor rate. This occurred in strain 8 and possibly in other strains.

3. In the majority of cases the age class in the later generations remained approximately the same as in earlier generations. In accordance with our former observations, it therefore remained characteristic of certain strains. However, while our more recent records show still more clearly the tendency of tumors to appear later in life in strains with a low tumor rate and at an early age period in those with a high rate, certain variable factors may readily produce, especially in strains with a low tumor rate, changes in the age class to which undue importance must not be attached.

REFERENCES

- (1) LATHROP, A. E. C. AND LOEB, LEO: *Proc. Soc. Exper. Biol. and Med.*, 1913, xi, 34. *Jour. Exper. Med.*, 1915, xxii, 646, and 713.
- (2) FLEISHER, MOYER S. AND LOEB, LEO: *Jour. Cancer Research*, 1916, i, 331.

SOMATIC MUTATIONS AS A FACTOR IN THE PRODUCTION OF CANCER

A CRITICAL REVIEW OF v. HANSEMANN'S THEORY OF ANAPLASIA IN THE LIGHT OF MODERN KNOWLEDGE OF GENETICS

R. C. WHITMAN

From the Henry S. Denison Research Laboratories of the University of Colorado,

Received for publication, March 4, 1918

For nearly thirty years the phenomenon known by von Hanse-
mann's term, anaplasia, has been widely used among patholog-
ists to describe and explain the origin of cancer cells. Any well
established facts, therefore, which throw added light upon the
nature of the process of anaplasia itself and any possible relation
it may have to cancer genesis must be important and significant.

The term anaplasia has been, however, so loosely used that it
will be worth while to review at length von Hanse-
mann's theory in order that we may start with a clear conception of what the
word really means. The pleomorphism of cancer cells and the
irregular, atypical mitoses so characteristic of their nuclei had
been observed and carefully studied since the middle of the nine-
teenth century. Arnold (Virch. Arch., vol. 79, cited by von
Hanse-
mann (1)), observing irregularities in the mitotic process
itself, had inferred that these might be of fundamental significance.
A voluminous literature dealing with the mitoses in cancer cells
and other forms of rapidly proliferating tissues had already ac-
cumulated when von Hanse-
mann (1) undertook a further inves-
tigation in the same field. In a carcinoma of the larynx he
found besides a great abundance of normal mitotic figures, large
numbers of tripolar and multipolar divisions and other departures
from the normal type already observed and described by earlier
writers; and in addition many hyperchromatic and hypochromatic
mitoses, the hypochromatism consisting not merely in a reduction
of the amount of chromatin in the chromosomes but rather in an

actual reduction of the total number of the chromosomes. He reproduces a monaster with only nine chromosomes. Further search led to the finding of a hypochromatic diaster one member of which contained five chromosomes, the other eight, or possibly nine. This asymmetry of division, which he regarded as analogous to the process by which he believes that differentiation, or specialization of the cells occurs during normal embryonic development, might, he thought, take place either as the result of delay, or of complete failure of the chromosomes to split longitudinally during the mitotic process; or else through failure of the two longitudinal halves of the chromosomes to separate and pass to the opposite ends of the spindle. In one of his cases he found numerous instances in which, in the monaster stage, late in the metaphase, (or telophase), the chromosomes were found lying in pairs close to and parallel with one another, indicating that splitting had occurred shortly before. Since splitting normally occurs early in the anaphase, he inclines to the view that the asymmetry in question is due to delayed splitting, at least in some instances.

In the search for further unequal mitoses von Hanseemann found, in a rodent ulcer, a diaster one member of which contained eleven chromosomes, the other sixteen. The fact that here, as also probably in the first case, the number of chromosomes in one or both members of the diaster is odd, constitutes further evidence that the asymmetry is due to the failure of splitting to occur at all in certain chromosomes, so that parts (of the chromosome) properly belonging in one-half are retained in the other.

No further mitoses were found in which inequality could be determined by actual count, but he was able with some patience to find, in every case, mitotic figures, both diaster and pluripolar forms, in which asymmetry was obviously present although the actual count could not be made. In a later paper (2), additional instances of this type are described and figured.

The occurrence of asymmetrical mitoses is then taken as established. I may add parenthetically that no one (so far as I can learn) who has concerned himself with this matter since has denied their occurrence. Ströbe (4), (cited by Hanseemann

(3)), indeed, claims to have observed such mitoses in a great variety of inflammatory and regenerative conditions, in sarcomata and benign tumors, and denies that they possess any biologic significance; but v. Hansemann contends that these assertions are based on inaccurate observations.

Now, since the cells with a reduced number of chromosomes can have no other possible origin than the tumor cells, it follows that the production of such hypochromatic cells (von Hansemann, as already mentioned, applied the term hypochromatism only to cells with a reduced *number* of chromosomes—not to cells with merely a reduced *amount* of chromatin) must be accompanied by the production of cells hyperchromatic to whatever extent the former are deficient in chromosomes. The tumor thus contains cells of three sorts, viz., cells containing an excess, a normal number, and a reduced number of chromosomes, between which there is no sharp dividing line. Only the extreme cases are recognizable. The last mentioned are capable of further mitoses, but sooner or later undergo a process of necrobiosis, as is evidenced by the occurrence of certain abortive forms of mitosis (2) which we need not discuss here.

The first type, on the other hand, may be assumed to convert itself into the resting forms of the tumor cell. Such cells may continue thereafter to divide symmetrically, or may undergo further asymmetrical divisions from time to time. Hansemann (3) states explicitly that asymmetrical mitoses may possibly, even probably, occur in other than tumor tissues, and cites cases from the literature. But he maintains that no epithelial cancer occurs without asymmetrical mitosis.

In order to make clear von Hansemann's idea of the significance of this departure from the normal type of mitosis it will be necessary to follow him in a digression. The biologic properties of cells are inherent in certain definite, formed constituents of the cells, larger than a molecule, which by growth and division transmit those properties to the descendants of the cells. Hansemann believed, in 1890, with de Vries, that these constituents, then called *idioplasmae* or *pangenes*, now called *genes* or simply *factors*, are seated partly in the chromosomes, partly in the cytoplasm.

They are now known to lie exclusively in the chromosomes (vide infra). The change in views on this point does not affect the validity of what follows. During embryonic development each new stage is marked by the appearance of cells not identical with the cells from which they arise. The production of such cells is identical with the process of differentiation of the several organs, and is accompanied by a division of labor among the several kinds of cells whereby each becomes more and more dependent on the coöperation of all the rest for its own continued existence, and becomes therefore less and less capable of independent existence. This division of labor, or *altruism* as he calls it, he regards as brought about (see especially his monograph (5)), by a process of unequal, (i.e., asymmetrical) division, so that one set of functions passes into one daughter cell, and another set into the other. But the separation of functions is not absolute and complete, as otherwise even the known limited powers of metaplasia, whereby cells of one type may be converted into other but closely related types, would be incomprehensible. Those idioplasmae, or factors, which dominate the character of the fully differentiated cell, he calls (5) *chief plasmae* (*Hauptplasmen*) and those factors which become latent, so as to exercise no visible effect on the functional activity of the cell under normal circumstances, he calls *secondary plasmae* (*Nebenplasmen*). The whole process by which the embryonic cell develops into the fully differentiated cell, he calls *prosoplasia*. The reverse process, by which a cell loses more or less completely its specialized functions, and its altruistic relation to the rest of the organism, i.e., gains in its capacity to live independently, he calls *anaplasia*.

It is at this point that von Hansemann's argument becomes a bit involved. Anaplasia is defined as a process of undifferentiation. Ribbert (6, p. 451 ff.), who comments on the frequent misunderstanding of von Hansemann's theory, himself falls into obvious error. von Hansemann thinks, he says, that "through the loss in differentiation, changes arise in the cells which he includes under the designation 'anaplasia.'" That is incorrect. Anaplasia means, not changes due to undifferentiation but the undifferentiation itself. Furthermore, von Hansemann is ex-

plicit (5) in his statement that the anaplastic cell is not an embryonic cell in the sense that it is identical with any cell at any time present in the normal embryo. Anaplasia is not a reversal of the normal direction of ontogenesis. The embryonic cell is a prosoplastic cell, and anaplasia begins where the embryonic cell leaves off. Anaplasia produces a cell different from any cell at any time normally present in the body.

The difficulty in understanding just what is meant by anaplasia arises primarily from hasty reading, but in large part also, it seems to me, from an unfortunate selection of descriptive terms on v. Hansemann's part. For, after all, one can hardly be blamed for visualizing *undifferentiation* as precisely that reversal of the normal course and direction of ontogenesis which anaplasia is not. If he had spoken of anaplasia as a process of *re-differentiation* much of the misunderstanding would have been avoided.

The asymmetrical mitoses then, according to von Hansemann, since the smaller of the unequal pair of daughter cells sooner or later dies, while the larger becomes the tumor cell, result in the production of cells whose chromosomes vary in number from cell to cell. Some of the tumor cells contain, indeed, an increased number of chromosomes and von Hansemann thinks (2) that it is the function of the pluripolar mitoses so often found to restore the normal number. Every loss of a chromosome, or group of chromosomes, involves the loss, on the part of that cell and its descendants, of those powers and functions the plasmas or genes for which were carried by the lost chromosomes; and, per contra, the retention in one cell of chromosomes which normally should have passed to another cell, involves the heaping up, the doubling, of all genes carried by the chromosomes thus retained. This cell, the cancer cell, is thus a "new kind of cell." In modern terminology it is, strictly and literally, a mutated cell. Since the process is, or at least may be, repeating itself from time to time, and here and there, in a tumor, it follows that the tumor cells themselves are by no means all alike in their biologic properties; that, on the contrary, an ever recurring process of mutation is taking place, with a tendency, however, to deviate more and more from the normal type. This explains why metastatic tumors,

for example, are often more, but never less, malignant than the primary tumor, as well as other related phenomena of tumor growth.

The anaplastic cell then is one in which, through some unknown agency, a progressive disorganization of the mitotic process occurs, which in turn results in the production of cells that are *undifferentiated* in the sense that those functions last to be acquired, most highly specialized, and perhaps most dependent for their continued functional efficiency on a continued altruistic relation to the other body cells, are *more or less* lost; but *re-differentiated* in the sense that the cancer cell is not at all an embryonic cell, but is a new biologic entity, differing from any cell present at any time in normal ontogenesis. But it must be remembered always that this new entity displays no characters absolutely and completely lacking in the mother cell—no characters created *de novo*, and out of nothing. Its changed behavior depends on exaltation of some qualities, and depression of others, all at least potentially present in the mother cell.

I would pause here merely to observe that numerous other theories of cancer genesis, Hauser's (7) "new cell-race," Butlin's (8) conception of the cancer cell as being itself the parasite of cancer, Oertel's (9) theory of the double nature of the nucleus, etc., to which references will be found in the bibliography, are merely variants of von Hansemann's parent idea, and differ from the last chiefly in terminology.

von Hansemann makes no attempt to account for the invasive tendency of malignant tumors beyond calling attention to the motility of cancer cells, first observed, he says, by Grawitz, and subsequently confirmed by many observers, including himself. It is also conceivable that the production of metastases and invasive growth depends merely on the passive transport of cancer cells in the body fluids. Nor does he make any attempt to explain the ultimate etiology of cancer.

Ribbert (6) opposes von Hansemann's theory on the following grounds:

1. As already mentioned Ströbe, on insufficient evidence according to von Hansemann, asserts that he has observed asym-

metrical mitoses in a great variety of non-cancerous lesions. Granting, for the sake of argument, the accuracy of Ströbe's observations, it does not follow that asymmetry must have the same significance in all cases. The complexity and delicacy of the structures concerned is so great that, as we now know (*vide infra*), the most varied results might easily follow an interference with the mitotic process, depending on differences in the character and seat of that interference which we are utterly unable to detect by direct observation.

2. On the point that anaplasia means not merely the return to an earlier stage of development but a loss of differentiation such that the cell becomes something which normally is found in the body at no stage of development, Ribbert contends that the very polymorphism and variation in size and structure of the nucleus, indicate that the cancer cell is not a cell which has attained to a characteristic condition through anaplasia. The cancer cell must, he urges, be a definite fixed entity (*etwas Eigenartiges sein*), i.e., it can not be a variable, a lawless thing. On the contrary its polymorphism arises simply from its abnormal environment.

To this it may be answered that there is, of course, absolutely no proof that all the cells of a given cancer have a like constitution; and since we would all agree with Cicero, that whatever is has a cause, we would concede that *something* in the environment is responsible for the change in the cell; but this something, insofar as it applies merely to the effect of environment (pressure, food, oxygen, etc.) on the behavior of the cancer cell in the fully developed tumor, is irrelevant except that such conditions might act to induce or accelerate further anaplastic changes. So far as it concerns the effect of the environment in setting in motion the initial anaplastic change, it is a question of etiology again quite irrelevant to von Hanseemann's argument.

3. In any except the most atypical and malignant cancers the degree of departure from the normal type is not great enough to furnish a basis for the concept of anaplasia. von Hanseemann (10) has himself pointed out that the loss of function in cancer cells is not often as complete as the generally accepted notion

would imply, and insists that the whole process of anaplasia is a gradual and progressive one, and that the loss of function increases *pari passu* with the anaplasia. Ribbert appears to have reached the point of view just indicated by visualizing von Hansemann's concept of the anaplastic cell as a cell exhibiting an extreme departure from the normal type, and excluding cells showing minor degrees of departure—an attitude quite out of harmony with the whole course of v. Hansemann's reasoning. Ribbert apparently thinks of anaplasia as a return to an earlier stage of embryonic development. von Hansemann thinks of it as a process carrying the cell in some entirely new direction—a direction, moreover, which is not the same in all tumors, nor even constant in the same tumor. Ribbert's total failure to grasp this point is clearly seen in the following paragraph (p. 455):

Whatever striking peculiarities (which were absent in the earlier beginning stages) the cancer cell in the fully developed tumor may show, are the result of changes occurring in the fully developed carcinoma; and whatever deviations from the normal type are present at the very beginning of cancer development, represent merely a loss of differentiation, such as we see also under other conditons. *There is no justification in either case for inferring that anaplasia has occurred.*

v. Hansemann constantly uses the term anaplasia interchangeably with undifferentiation, except that, as above noted, anaplasia implies undifferentiation in some new direction or directions.

4. Anaplasia offers no explanation of the invasive growth. A mere tendency to increased growth, due to anaplasia, would cause merely a local hyperplasia, in which the cells would grow in the direction of least resistance, or toward the free surface. Ribbert assumes that the inflammatory changes always present in the underlying connective tissue loosen the latter and thus favor, mechanically, infiltration by the tumor cells. But this raises a double difficulty. The assumption that inflammatory changes favor rather than hinder the cancerous invasion sharply challenges our concept of the biologic significance of the inflammatory reaction. Ribbert meets this by arguing that since the reaction

is excited by cell products not foreign to the body it remains sluggish and lags behind, in intensity, its exciting cause.

What we chiefly see in a primary carcinoma is a cellular infiltration and moderate proliferation of the fixed elements. These processes are not adequate to check an epithelial infiltration; on the contrary they loosen the connective tissue, and thus create a lowered resistance, a sort of cavity formation, which sets in motion the downgrowth of the tumor cells (p. 493).

But it is at least difficult to understand how any conceivable change in the connective tissue can ever lower the resistance offered by it to a point less than that on the free surface. We have to assume that, if the connective tissue is the prime cause of the invasive growth, it is so by virtue of an actual positive attraction, not merely a negative lowering of the mechanical resistance: which brings us back to the starting point where our ideas of the fundamental significance of the inflammatory process are violated.

5. And finally, in his discussion of Hauser's new cell race theory (p. 458), Ribbert raises a point which applies with equal force to von Hansemann. The cancer cell cannot be a "new race of cells," because, "How can a cell arise which lies *entirely* outside the phylogenetic series? How can cells acquire *entirely* new properties?" (Italics mine.) I have urged already that the cancer cell exhibits no *entirely* new properties. Further he says "All development is continuous. If, in the course of phylogenesis, new sorts of cells are produced, they are produced always gradually, and are never elements injurious to the organism"—a statement which Ribbert would hardly make save in the heat of an *ex parte* argument. And again (p. 477), he maintains that new biologic properties arising in the course of ontogenesis can only arise in accordance with fixed laws of inheritance; that if we assume that properties arise independently of such laws, they can do so only as the result of adaptation to changes in the environment. Applied to tumors, this means that tumor cells can arise only under those influences which determine tumor formation. Under no conceivable circumstances could cells learn to

live under abnormal conditions until after those conditions have acted upon them. Stimuli call forth in cells and tissues, it is true, insofar as these are not injured, adaptive changes, but the adaptation is to the stimulus, not to something else, hence not to the cause of the invasive growth. Stimuli therefore cannot produce destructive properties in the cells. The ability to live in other tissues, and to invade them, could only be acquired under the conditions existing during the growth itself. Hence a primary change in the cell leading to invasive growth cannot exist.

Now it is true that mutations can arise only as the result of some change or other in the environment. To assert otherwise would be to assert that things happen without a cause. But it is not true that the net result of the adaptive change is never injurious to the organism. Are hemophilia, and Daltonism, and intrinsic epilepsy advantages or disadvantages? Yet these heredity conditions must in every case have arisen in the first instance as a mutation in response to some change in the environment. Nor is it true that the adaptive reaction always stands in direct and obvious relation to the environmental change which produced it. The reaction may be excessive or involve remote effects quite different from the primary purpose to be served. If I may be permitted to cite examples outside the domain of heredity (if they are outside), we should, I dare say, all agree that the inflammatory reaction is on the whole designed by nature to serve a beneficent end, but the violence of the reaction in a pneumonia may render the reaction itself more dangerous to life than is the cause of the reaction; and again, the healing of multitudinous minute injuries in the kidney leads finally to the death of the patient. The error has a double source: Ribbert is obsessed with the idea that adaptation must always be protective and must always show a direct and obvious relation to its cause; just how erroneous this is will become apparent from what follows. And secondly, he entirely fails to keep separate in his own mind two distinct issues. He himself is chiefly concerned with the factors that lie back of and induce the cancerous change in the epithelial cell; von Hansemann, on the other hand, is interested in the intracellular mechanism by which that change comes into being.

Before I proceed I should like to pause long enough to point out that, if the distinction just drawn be kept clearly in mind, no irreconcilable difference is found to exist between Ribbert and von Hansemann. Each has for thirty years vigorously and sometimes heatedly championed his own view as opposed to that of the other. Yet, except perhaps in certain details, neither view excludes the other.

Morgan (11) and his associates have observed more than 125 mutations occurring "spontaneously" under the uniform conditions of laboratory cultivation, in the fruit-fly, *Drosophila ampelophila*. This fly has three pairs of autosomes and one pair of sex chromosomes. The last differ morphologically from the



FIG. 1. CHROMOSOMES OF THE *D. AMPELOPHILA*

Female at the left, male at the right. The X chromosomes at the bottom appear as straight rods. The XY complex in the male at the right is recognizable by its different morphology. Figures 1 and 2 are reproduced by permission of the author from Morgan's "A Critique of the Theory of Evolution," Princeton University Press, 1916.

autosomes, and, in the male, from each other, the female having typically two "X" chromosomes, and the male one "X" and one "Y" chromosome. The "Y" chromosome is not the carrier of any known hereditary factors, and indeed has no known function beyond the fact that males lacking it are sterile (fig. 1), (Bridges, 12). By methods the description of which may be omitted here, it was shown that the factors of inheritance, or genes, have their seat in the chromosomes; that the several genes are arranged in a row, or rows, in the chromosome, like beads on a string; and that each gene has a definite fixed locus in the chromosome, which can be determined and mapped (fig. 2). I select for comment a few mutations which illustrate points with which we are concerned. Among these is the character *eyeless* (fig. 2)

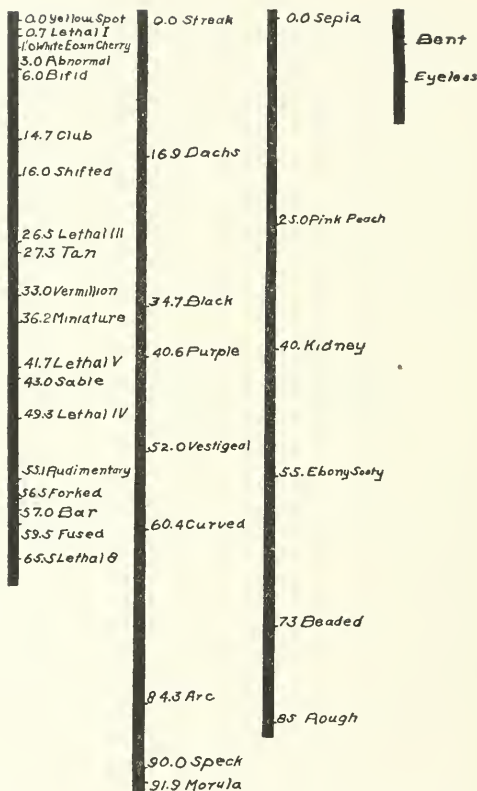


FIG. 2. CHART OF THE CHROMOSOMES OF *D. AMPELOPHILA*, SHOWING THE BEST KNOWN OF THE MUTATIONS OBSERVED

The figures are the unit distances apart, and the words are the laboratory designations of the several mutations. The chromosome at the left is the X chromosome and the short chromosome at the right is the fourth chromosome indicated by the pair of dots in figure 1 (Morgan).

in the fourth chromosome. This change took place at a single step, but is variable, some of the individuals showing more or less rudimentary organs. Morgan comments (p. 67):

Formerly we were taught that eyeless animals arose in caves. This case shows that they may also arise suddenly in glass milk bottles, by a change in a single factor.

There also appeared in the cultures some twelve modifications of the shape and size of the wings, which might easily be arranged in a series culminating in a wingless insect. But as a matter of fact each of these arose independently of the others and was shown to be due to distinct factors. Five of these have their loci in the first chromosome, and seven in the second chromosome. Moreover factors not infrequently produce more than one effect. For example,

A mutant stock called "rudimentary wings" has as its principal characteristic very short wings. But the factor for rudimentary wings also produces other effects as well. The females are almost completely sterile, while the males are fertile. The viability of the stock is poor The hind legs are also shortened.

Again the factor producing one of the wing changes above mentioned is a dominant so far as the shape of the wings is concerned and is at the same time a recessive lethal factor. As this character is sex-linked, no males carrying this factor (they being haploid as regards their sex chromosome) survive. In another case a factor causing a difference in the body color, called tan, also produces a loss of positive heliotropism.

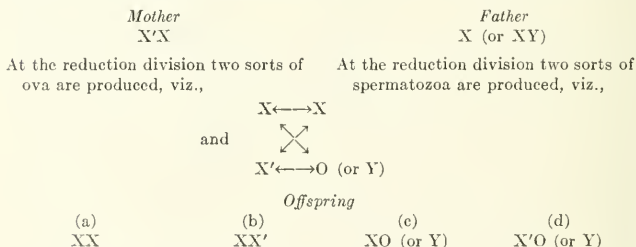
A gene which causes duplication of the legs was found and it was further shown that this effect takes place only in the cold. Flies grown at ordinary temperature have normal legs. In another culture a number of flies were found whose wing pads failed to expand, and two bristles were lacking on the side of the thorax. It was found that the wing anomaly was present in only 20 per cent of the flies of this strain, apparently because it was an "environmental effect peculiar to the stock," whereas the absence of the bristles was constant. Thirty different

factors were identified which have an influence on the eye color. "It is probable that there are at least as many normal factors that are involved in the production of the red eye of the wild fly."

A considerable number of lethal factors (eight including those listed by Bridges (12)) has been located in the X chromosome. One of these, lethal 7 (Bridges, loc. cit.),

causes the death of the individual at the mature larval stage. Those larvae which are about to die can be separated. . . . because when half mature [they] leave the food and wander about on the surface of the culture bottle. Furthermore, while still young, one or more intense black specks appear in their body cavities. As the larvae become older this character becomes more conspicuous, so that one can easily pick out those cultures in which the mother was heterozygous for lethal 7 Lethal 7 is then a larval character of such virulence as to cause a change in the instincts of the individual and finally cause its death.¹

¹ It is not without interest in this connection to note that at least one lethal factor is known in man. Daltonism and hemophilia are sex-linked characters, and Daltonism, according to Rössle (13) when total is, in the male, recessive; when partial, dominant; in the female, it is recessive. This is not the case, as Morgan shows, for according to von Winniwarter (14) the constitution of the sex chromosomes is XX in the female, X in the male; according to Wieman (15) the constitution of the human sex chromosomes is XX in the female, XY in the male, exactly like the *Drosophila*. The mechanism of transmission is the same in either case, as follows:



The X' is the sex chromosome carrying the recessive factor for Daltonism. The arrows indicate the possible combinations of ovum and spermatozoon in fertilization.

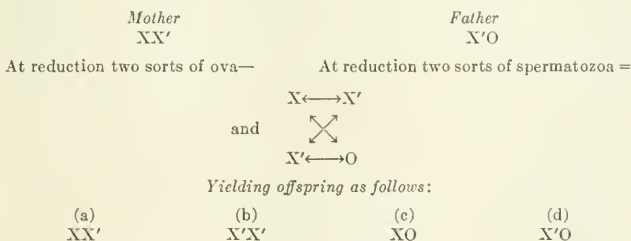
It is apparent that half the daughters will be normal (a); half will be apparently normal but like the mother, capable of transmitting the condition (b), since the

Bridges (12) also describes, and Morgan (11) offers positive cytological proof of, the occurrence of non-disjunction, i.e., the failure of homologous chromosomes to separate, in mitosis, to the opposite poles of the spindle, with the resulting production of unexpected Mendelian results. This, it will be recalled, is precisely one of the mechanisms by which von Hansemann conceived that anaplasia might occur.

Among the factors most significant and important to the present discussion are the *modifiers*. Morgan cites a number of cases to show that so-called *unit characters* are not infrequently the net result of the interaction of several genes, and that in

effect of the recessive factor is offset, so far as that individual is concerned, by the corresponding dominant allelomorph in X; half the sons will be normal (c) and half will be color blind (d), since, whether a Y chromosome is present or not, no dominant allelomorph is present to offset the recessive for Daltonism in X¹ (v supra, p. 139).

A color blind female can only be produced by crossing a female transmitter of Daltonism like the mother above with a color blind male, as follows:



a = apparently normal transmitter like the mother

b = color blind female

c = normal male

d = color blind male

The male is haploid as to the sex chromosomes; the female is diploid, hence sex-linked recessive characters behave like recessives in the female, but like dominants in the male.

Hemophilia is exactly on all fours with the above, except that, according to Rössle (loc. cit. p. 36) "The occurrence of genuine hereditary hemophilia has never yet been demonstrated in women." Since color blindness does occur in women, the failure of hemophilia to occur can only be explained on the assumption of a miraculously happy chance, or on the assumption that the hemophilic gene, when doubled, acts as a lethal.

some cases a factor is entirely without effect except in the presence of other factors (p. 163, et seq.).

And finally Bridges (16) has shown that a definite region of the X chromosome, including the loci of several factors, may entirely disappear, may become, in other words, "genetically non-existent," and that this loss when present in the (haploid) male or in both X chromosomes of the (diploid) female may render the individual non-viable. He says,

In general it is to be anticipated that cases of deficiency should act as lethals, since it seems probable that any very extensive piece (of a chromosome) includes a locus vital to the animal.

Furthermore, it has been shown by Bridges (12) that an entire chromosome or a part of a chromosome may be lost when a chromosome "lags" on the spindle during mitosis, though why a chromosome should lag behind its fellows is of course not known. It can only be surmised that, through some disturbance, the character of the chromosome substance undergoes a change, analogous, perhaps, to the change undergone by endothelial cells in inflammation, whereby the cell, although it shows no visible alteration, opposes a greatly increased frictional resistance to the flow of the blood. If such a change does take place we may imagine the affected chromosome as adhering to the spindle and thus lagging behind because it is, so to speak, mired. A similar process was described by von Hansemann (*loc. cit.* 5) as occurring in cancer cells, and was thought by him to constitute an additional mechanism by which anaplasia might occur.

It has then been proved, on the one hand (Morgan, Bridges), that changes in the chromosome of an exceedingly delicate character may produce profound and far reaching changes in the structure and character of the individual; and that such changes arise without apparent cause, without waiting upon any visible changes in the environment. Roughly the law of heredity, so far as the initiation of mutative changes is concerned, lies, so far as can be observed, in their very lawlessness, though of course actually that is not the case. On the other hand, von Hansemann has furnished conclusive evidence that the cancer cell undergoes

changes in its chromosomes of far greater extent and severity than the slight changes shown to produce mutations. It may, I think, be regarded as proved that the changes in the cancer cell described by von Hansemann are precisely of the character demanded by the available data to explain the change in behavior of the cancer cell as compared with the normal parent cell. The trouble is, indeed, not that the changes observed in cancer cells prove too little, as Ribbert argues, but that they seem rather to prove too much. For example, Bridges' remark, already quoted, that "in general it is to be anticipated that cases of deficiency should act as lethals, since it seems probable that any very extensive piece includes a locus vital to the animal," raises a difficulty here, since the cancer cell is obviously enormously deficient in Bridges' sense. But Bridges is speaking of inheritable changes, i.e., of modifications presumably involving equally every cell of the body. Such a change, when it involves a "locus vital to the animal" would obviously have quite a different significance when it involves a limited group of cells. Nor need such a change be lethal to that group of cells, especially when those cells, by the very nature of the mutation, have acquired the greatly increased power to stand alone.

But clearly anaplasia, as I said, proves too much. I incline strongly to the view that we are on safer ground in insisting, not on the loss of entire chromosomes and groups of chromosomes which anaplasia is said to produce, but rather upon the profound general disturbance of the mitotic process which is obviously present, and the abundant opportunity for modification of factors, and the production of new factors, which is thus afforded. For if von Hansemann's explanation of asymmetrical mitosis as due to delayed or absent longitudinal splitting be correct, it is apparent that the smaller of the asymmetrical pair of cells would contain only those chromosomes which have split in due season; and the larger of the pair would contain the homologue of those chromosomes, plus all those chromosomes which have failed to split. Since the larger cell is assumed to be the tumor cell, the net result of the process would be, not a loss of hereditary factors

to the tumor cell, but a heaping up, a doubling, of all those that remain behind during mitosis, in the tumor cell.

I quote here a remark of Morgan's (loc. cit., p. 162), .

By crossing different wild species, or by crossing wild with races already domesticated, new combinations have been made. Parts of one individual have been combined with parts of others, creating new combinations. *It is possible even that characters that are entirely new may be produced by the interaction of factors brought into recombination.* [Italics mine.]

This brings us to the chief difficulty, viz., the question whether a somatic mutation can occur, i.e., a mutation involving only somatic cells, and not the germ cells, therefore not inheritable. Most of the observations reported on this point have to do with plants—which makes it important to remember that the mechanism of Mendelian inheritance is identical throughout the plant and animal kingdoms. Cockerell (17) has described a deficiency in the rays of a sunflower, involving equally and in the same relative position, all the flowers produced by the plant, which he interprets as a somatic mutation. But no experiments were made to determine whether the mutation was purely local, or involved the constitution of the whole plant. A somewhat different condition is presented by the "bud variations" occurring in plants, and consisting in the production of unlike flowers on different branches of the same plant, e.g., a yellow chrysanthemum carrying white flowers on one branch. A recent discussion of this phenomenon is that of East (18). It is obvious that, since the color of the flower is known to depend on definite factors, the development of a branch carrying flowers of a color different from that of the flowers on the other branches could only arise through the failure of the factor to pass over, during mitosis, into the cell from which that branch developed. The branch, in other words, represents a somatic mutation due to asymmetrical mitosis. Nor are similar instances lacking in the higher animals. Every one has seen dogs and horses whose eyes differ in color. The color of the eye is known to depend on Mendelian factors. There is therefore no other conceivable explanation than that the

anlage of the lighter eye has "dropped" the color factor, or the darker eye has "picked up" a color factor present or absent, respectively, in the other eye. Clearly also this is a somatic mutation, based on asymmetrical mitosis. Certain anomalies of skin pigmentation may also be cited here, though, it must be admitted, on less secure grounds. But, since the production of melanin in the skin, as in the eye, depends on Mendelian factors, the disappearance of normal pigment in vitiligo, in certain scars, etc., can be most plausibly explained as due to the dropping of that factor; and, on the other hand, an excess of pigment, in naevi and some scars, may be due to the picking up of pigment genes. Where the pigment is more or less symmetrically disposed, as in freckles, which are listed by Rössle (*loc cit.*) among the hereditary anomalies of the skin, there is of course less ground for assuming a purely somatic variation, and in Addison's disease the pigmentation seems to depend on a disturbance of metabolism which may or may not involve a change in the Mendelian factors for color.

I would not be understood as maintaining that the status of all the preceding pigment anomalies as somatic mutations is proved, though I believe that the status of those referred to in the eye is fairly clear. Granting that conclusive evidence of the occurrence of somatic mutations in man is lacking, I submit that no inherent impossibility, or even improbability, of such an event exists. Mutations must arise in the first instance by a change in the factors either of one or the other of the sexual cells which unite to produce the individual, or of the cell which is the product of that union. If such a change can take place in these cells there is no apparent reason why it cannot take place in a cell anywhere in the body. Those who believe that cancer is inheritable will, of course, experience no difficulty on the score of somatic mutation.

But cancer may be inheritable in quite another sense. It is a matter of common knowledge that highly hybridized, or mongrelized, plants and animals are prone not to breed true. The bud-variations in plants already referred to are much commoner in hybrids (*East, loc cit.*). Extreme examples are furnished by the potato and dahlia, neither of which can be bred true from seed.

In certain of the dahlias (I may mention the varieties known as *Mrs. H. J. Jones* and *Fritziman*), this instability is so great that flowers developing on the same plant often differ from one another to a greater or less extent. Parallel with this fact is the general impression that cancer is less frequent among aboriginal races than among the civilized and highly hybridized races. I have compiled the following table from Hoffman (19) showing the cancer rates for various races. In general the rates increase with the generally accepted degree of mongrelization. My first attempt to secure adequate confirmation of this impression quickly convinced me that the confirmation would involve such an extended discussion as to carry me beyond the possible limits of a single paper. I have therefore reserved this question for separate treatment. I would, however, call special attention to the marked difference in the cancer rate of the Chinese, Japanese, and ourselves; in that of the negroes in the United States and the native blacks of Johannesburg; the wide variations in the rates for the several races of Hungary listed in the table; and the tremendous difference indicated between the rate given for Iceland on the one hand and Finland and Switzerland on the other.

	Rate per 100,000
Chinese in Hongkong, 1901-1912.....	6.07
Europeans in Hongkong, 1901-1912.....	50.107
Japan as a whole, 1899-1911.....	44.0 to 66.9
United States as a whole, 1900-1916.....	63.0 to 81.8
Negroes in the United States, 1910-12.....	56.1
Johannesburg, South Africa, 1909-1911:	
Whites.....	51.9
Asiatics.....	46.5
Native blacks.....	14.5
North American Indian.....	"extremely rare"
Constantinople, 1908-1912	
Mohammedans.....	22.6
Jews.....	44.2
Armenians.....	56.3
Greeks.....	56.1
Others.....	46.7
Austria, 1907-1911:	
Serbo-Croats.....	31.3
Polish Ruthenians.....	33.8
Slovene.....	47.9
Bohemian.....	102.0
German.....	110.0

Hungary, 1901-1904:	
Ruthenian.....	4.6
Rumanian.....	11.3
Croats.....	17.9
Serbian.....	19.3
Slovak.....	28.5
Magyar.....	44.1
German.....	56.4
Others.....	32.5
Iceland, 1908:	
Males.....	15.0
Females.....	39.7
Finland, 1909.....	100.0
Switzerland, 1881-1912.....	109.7 to 125.9
Italy, 1900-1912.....	52.2 to 64.7
Spain, 1900-1912.....	39.3 to 55.5
Portugal, 1902-1910.....	stationary at 22.7

It is possible, of course, that a part at least of the apparent variation is due to variation in the proportion of cases which escape diagnosis. But this possibility would seem to be unimportant in such regions as Hungary, and perhaps Constantinople. Caution is also needed to avoid drawing inferences from a mere post hoc propter hoc argument. But in view of the considerations already urged, the table is at least suggestive. The situation throws an interesting light and affords biologic justification for the instinct of race purity, if we assume that the inter-marriage of races, by introducing a great mass of new factors of heredity, introduces also an element of instability by which the tendency to cancer is increased.

Finally I wish to acknowledge my debt, and express my gratitude, to my colleague, Professor Cockerell, of our department of biology. His suggestion started me in the course of reasoning which led to the foregoing essay, and he has constantly aided me with suggestion and advice, in dealing with the biological questions involved. I had hoped that he would join with me in authorship, but he has, modestly, or prudently, declined that responsibility.

REFERENCES

- (1) VON HANSEMAN: Virchows Arch., 1890, cxix, 299.
- (2) VON HANSEMAN: Virchows Arch., 1891, cxxiii, 356.
- (3) VON HANSEMAN: Virchows Arch., 1892, cxxix, 436.
- (4) STRÖBE: Cited by von Hanseman. Ziegler's Beitr., 1892, xi, 10.
- (5) VON HANSEMAN: Studien über die Spezificität, den Altruismus, und die Anaplasie der Zellen, Berlin, 1893.
- (6) RIBBERT: Das Karzinom des Menschen, Bonn, 1911.
- (7) HAUSER: Das Zylinderzellen Karzinom, Jena, 1890; Virchows Arch., 1895, cxli, 485.
- (8) BUTLIN: Lancet, London, 1911, clxxxi, 1535.
- (9) OERTEL: New York Med. Jour., 1907, lxxxvi, 14.
- (10) VON HANSEMAN: Ztschr. f. Krebsforsch., 1906, iv, 565.
- (11) MORGAN: Critique of the Theory of Evolution, Princeton University Press, 1916.
- (12) BRIDGES: Genetics, 1916, i, 1 and 107.
- (13) RÖSSLE: Aschoff's Path. Anat., 1911, i, 35.
- (14) VON WINNIWARTER: Arch. d. Biol., 1912, xxvi, 91 and 189.
- (15) WIEMAN: Am. Jour. Anat., 1917, xxi, 1.
- (16) BRIDGES: Genetics, 1917, ii, 445.
- (17) COCKERELL: Jour. Heredity, 1917, viii, 467.
- (18) EAST: The Amer. Naturalist, 1917, li, 129.
- (19) HOFFMAN: Mortality from Cancer throughout the World, Newark, 1915.

THE COST OF CANCER IN NORWAY¹

F. G. GADE

Received for publication, May 18, 1918

The various disasters to which death and disease give rise have an economic side, which occasionally makes itself strongly apparent. The author has therefore tried to calculate the economic losses to Norway brought about by deaths from the cancerous diseases (carcinoma and sarcoma) in that country.

The calculations are based on one side upon the mortality statistics of Norway for the years 1902-1911, reporting 22,093 deaths caused by malignant tumors; and on the other, upon the valuation of Norwegian lives in 1912, as published by Mr. A. N. Kiaer in *Statsoekonomisk Tidsskrift*, 1913.

Mr. Kiaer, then director of the Norwegian Statistical State Bureau, took for his starting point the average wage and income-tax within all classes and ages of the population, and calculated from these data the capital values of individual lives at the various ages, by discounting the probable income during the remainder of life.

As these valuations were calculated for 1912, however, the results would give too high values when applied to the period 1902-1911, for wages, and consequently the value of life in Norway during this period, have risen by at least 1 per cent a year; it has been necessary, accordingly, to reduce Kiaer's results to the standard of 1906, representing the medium year of the decade 1902-1911.

Concerning the age and sex distribution of the 22,093 deaths from malignant tumors, columns 1 and 4 of the subjoined table will give necessary information. It may be mentioned that

¹ This paper is an abstract, by the author, of one published by him in *Norsk Mag.f. Lægev.*, March, 1918, page 325.

the number of deaths at different ages during single years shows only slight variations throughout the period under discussion.

Loss of capital through cancer mortality 1902-1911

Age	MALES			FEMALES		
	(1) Number of deaths 1902-11	(2) Individual capital value (1906)	(3) Loss of capital	(4) Number of deaths 1902-11	(5) Individual capital value (1906)	(6) Loss of capital
		<i>kroner</i>	<i>kroner</i>		<i>kroner</i>	<i>kroner</i>
0-5	52	4,175	217,100	25	2,437	60,925
5-10	20	5,882	117,680	16	3,424	54,784
10-15	13	7,882	102,466	19	4,545	86,355
15-20	36	9,774	251,864	32	5,375	172,000
20-25	29	11,126	322,654	32	5,788	185,216
25-30	57	11,941	680,637	81	6,000	486,000
30-35	78	11,908	928,824	138	5,971	823,998
35-40	173	11,435	1,978,155	292	5,658	1,652,136
40-45	300	10,628	3,188,400	546	5,104	2,786,784
45-50	549	9,404	5,162,796	797	4,326	3,447,822
50-55	965	7,856	7,581,047	1,111	3,502	3,890,722
55-60	1,277	6,094	7,782,038	1,326	2,674	3,545,724
60-65	1,619	4,308	6,974,652	1,512	1,858	2,809,292
65-70	1,718	2,769	4,755,142	1,551	1,205	1,868,955
70-75	1,674	1,507	2,522,718	1,503	699	1,050,587
75-80	1,362	670	912,540	1,246	355	442,390
80-85	609	288	175,392	669	164	109,716
85-90	234	127	29,718	332	82	27,224
90-100	41	34	1,394	59	32	1,888
	10,806		43,685,217	11,287		23,502,522

Total kr. 67,187,739 = \$18,000,000.

In a former paper, a summary of which has been published in this Journal (1918, iii, 107) the author has shown that the cancerous diseases in Norway attack males and females in exactly the same proportion; also, that in both sexes the highest number of deaths from malignant tumors occurs between sixty to seventy-five years, during which period there are recorded nearly half of the total number of all deaths from cancer. This fact will be evident from the table above, which also shows that women from thirty-five to fifty are considerably more liable to cancer than men (cancer of the breast and the uterus in particular having

its maximum during this period); in men, the mortality is higher at sixty to eighty-five years (cancer of the stomach and of the skin and lips). For the ages above eighty-five years, the greater number of females surviving at this age explains the greater number of deaths from cancer in women.

As seen in columns 2 and 5 of the table, the capital value of an individual reaches its height in the years between twenty and forty, and particularly between twenty-five and thirty-five years. For males, this value has been calculated by Kiaer to be about kr. 12000 (= \$3,217, a dollar being equal to kroner 3.73); for females, about kr. 6000 (= \$1,609), the capital value of men amounting through all age periods to about twice as much as that of women, the worth of the household work performed by the latter having been taken into account by estimation. That this economic valuation of the various ages and the two sexes can by no means give a true and valid standard for the real worth of the individuals and the sexes, need not be specially pointed out; the individuals are here considered exclusively as income-producing members of society.

From these calculations we find, for the period in question, that the total loss by deaths from cancer is among

Men.....	kr. 43,700,000 =	\$11,700,000
Women.....	kr. 23,500,000 =	6,300,000
Total.....	kr. 67,200,000 =	\$18,000,000

Consequently it averages *for each single year* kr. 6,720,000 = \$1,800,000, for a population of scarcely 2,500,000 inhabitants.

This total, however, must be understood to cover only the direct loss of life-capital, and must be considerably increased if other sources of economic loss through the diseases in question are to be considered. Moreover, the constantly increasing number of deaths from cancer reported in Norway involves a correspondingly increasing loss. Again, it must be taken into consideration that among the 12.5 per cent of all deaths in Norway, the causes of which are not diagnosed, there will probably be found a proportionate number of deaths from cancer.

Finally, the rising wages and capital value of life in Norway will constantly augment the economic loss brought about by comparatively early death.

All things considered, *the total yearly economic loss through cancerous diseases in Norway may at present be estimated at about 10,000,000 kroner, or \$2,680,000.*

PRIMARY SPONTANEOUS TUMORS OF THE TESTICLE AND SEMINAL VESICLE IN MICE AND OTHER ANIMALS

XII. STUDIES IN THE INCIDENCE AND INHERITABILITY OF SPONTANEOUS TUMORS IN MICE

MAUD SLYE, HARRIET F. HOLMES, AND H. GIDEON WELLS

*From the Otto S. A. Sprague Memorial Institute and the Department of
Pathology of the University of Chicago*

Received for publication, April 8, 1918

Tumors of the testicle would seem to be uncommon in mice, for we have been unable to find even a single case reported in the literature, although they have been described in other species of lower animals. Thus, among 103 primary tumors observed by McCoy (1) among 100,000 rats killed in plague work, there was one described as an "angiosarcoma" of the testicle, without further details. Other series of tumors in wild rats (Woolley and Wherry (2), Beatti (3)) do not record cases of testicular tumors. Caspar (4) says:

Carcinomas of the testicle, often described in horses and dogs, form sometimes soft, sometimes hard tumors, in which not rarely single portions are formed differently. Through mucoid and colloid degeneration of the cell nests, cysts with gelatinous contents may be formed.

Infiltration along the spermatic cord and lymph-node metastases are often observed. Horses appear especially to develop testicular tumors, particularly if we consider the relatively small number of old animals that have not been castrated. In Japan, where this operation is less often performed than in Europe, equine testicular tumors are most abundant. Thus Kimura records the finding of 49 such growths among 142 tumors observed in 77,224 slaughtered horses. This may be compared

with the figures given in the census statistics on the mortality from cancer in the registration area of the United States, which shows that of 52,420 cases of malignant tumor there were but 121 recorded as arising in the testicles, or 2.3 per thousand; as 21,282 of the cancer cases were males, the proportion of testicle tumors is 5.8 per thousand of all tumors in males. Kimura studied in detail 12 specimens of equine "orchidoblastomas" varying in size up to 7500 grams weight; all were unilateral, and in at least five cases there were metastases in the spermatic cord and the inguinal and lumbar lymph-nodes. He describes their anatomic characteristics as follows:

1. Tumors usually develop without altering the normal shape of the testis. The albuginea is somewhat thickened, showing more or less engorgement of blood-vessels on its surface and expanding with the growth of the tumor, and may continue to enclose it even when of large size.

2. Generally the entire glandular portion of the organ is replaced by new growth, but sometimes the atrophied deep brownish glandular portion may remain under considerable compression at the peripheral part.

3. The tumor consists of nodules, which vary considerably in size and are generally surrounded and separated by strands of fibrous tissue in varying amount.

4. On section the cut surface of the tumor parenchyma is of medullary yellowish gray-white colour and of soft or somewhat firm consistence, whilst here and there creamy yellowish red-gray islets which represent the softened necrotic areas, and various-sized irregular deep red hemorrhages can be seen, so that these figures give an almost marble-like appearance.

5. The spermatic cord and the inguinal and lumbar lymph-nodes are frequently apt to become involved, and distant metastases perhaps may occur (dissemination in the peritoneal cavity).

Microscopical examination

1. Corresponding with the macroscopic appearance, bands of fibrous tissue are seen traversing the section in all directions. These vary in width and divide the parenchyma into islets of various sizes and shapes, giving an alveolar appearance.

2. The tumor cells are roughly polyhedral, spheroidal, or ellipsoidal in shape, the cytoplasm stains in general feebly with eosin and appears homogeneous or slightly granular, varying in size from $11\ \mu$ to $35\ \mu$ in diameter.

The nuclei are of three kinds, according to their shape, size, and staining qualities.

(i) Spheroidal or short ellipsoidal, deeply stained small nuclei with invisible nucleoli.

(ii) Ellipsoidal large nuclei with vacuolar appearance, in which only the nuclear membrane and nucleolus have taken on the stain, and the latter stains bright red with eosin (usually only one, sometimes two or three in number).

(iii) Nuclei which show intermediate characters between (i) and (ii).

The size of these nuclei varies from $6.5\text{--}16\ \mu$ in diameter. Multi-nuclear cells with strikingly large cytoplasm and a circular arrangement of their nuclei about a central lumen are numerous in all cases. Mitotic figures are abundant in the tumor cells, most of them being bipolar, though figures with three, four, or more poles, and cells with irregular distribution of chromosomes are not infrequently met with. Generally the tumor cells are rich in glycogen, but only a small amount of fat is to be seen; there are small areas of haemorrhage and necrosis in the tumor parenchyma, and the fat can be shown circumscribed especially in the latter, in sections stained with Sudan III.

3. Traversing the section in all directions and cutting up the parenchyma into islets, the fibrous stroma gives off finer strands—sometimes only a few fibers—running in towards the peripheral portion of the enclosed parenchyma from the main circumferential band; but the tumor cells lie along the walls of the alveoli without intimate connection with them and are packed together without fibrillar intercellular substance, so that not all the tumor cells are intermingled with the fibrous stroma. The stroma represents more or less round-cell infiltration, which mostly consists of lymphocytes; and reactive proliferation of the connective tissues may sometimes be seen. Elastic fibers appear generally in small amount.

4. In the atrophied residual glandular portion, spermatogenesis is usually not visible; only one case among four with surviving glandular tissue showed spermatogeny. The cells in the atrophic seminiferous tubules greatly resemble tumor cells morphologically. Interstitial tissue and membrana propria of the seminiferous tubules are in general

increased, and masses of granular cells in the interstitium (*Zwischenzellen*) appear atrophic.

As compared with these observations in Japan, Sticker (6), in his compilation of the European literature on the occurrence of tumors in the lower mammals, reports that of 298 malignant tumors in horses, but 11 were in the testicle; in cattle, of 110 tumors none were in the testicle, and there were none among the recorded neoplasms of sheep (7 cases), of cats (7 cases), or of swine (7 cases). But of 766 tumors in dogs, 18 were in the testicle. Of 305 cases of bovine tumor observed in the slaughter house at Glasgow, according to Trotter (7), there was none in the testicle. Undoubtedly these figures give an entirely false impression as to the frequency of testicular tumors in the lower animals, since so few old, uncastrated horses, cattle, swine, or sheep come to the slaughter houses, where most of the neoplasms are detected. According to Caspar (4) a testicular cancer has been observed in a cat by Leiserling, metastases having been found in the mesentery of the transverse colon. A case of carcinoma of the bovine testicle is mentioned without details by Murray (8), and Williams (9) says that in the ass sarcoma of the testicle has been described; he also mentions the occurrence of tumors in the testis of swine, but without citing the origin of these reports. Wolff (10), in his compilation of the literature, refers to several cases of testicular tumor in dogs and horses, but records none in mice or other animals. He refers to the case, reported by Axe, of a tumor arising in an ectopic testicle in a pony, and several other cases in the literature suggest that in dogs and horses, as in man, the ectopic testicle is especially likely to undergo malignant transformation.

One of us (H. G. W.) has observed two cases of primary tumor of the testicle in dogs, which, because of the infrequency of recorded cases in the literature, are briefly described here, as follows:

Case I. A pure blooded Pomeranian, age five and one-half years, was brought to the laboratory by Dr. J. W. Walker, in order that a growth involving the right testicle might be removed by operation.

This growth had been noticed at least two years before and had grown very slowly. The operation was done under anesthesia; the animal recovered and is now in apparently perfect health three years after the operation. No metastases could be palpated in the groin or elsewhere. The animal had been suffering from extensive loss of hair for some time before the operation, but after it the normal coat was restored. The growth was about 3 x 2 x 2 cm., involving the body of the testicle as an encapsulated nodule and leaving a compressed remnant of testicle tissue at one side. There was no infiltration, and the epididymis was not involved.

Microscopically it shows irregular coarse lobules with heavy fibrous tissue trabeculae, packed full of large cells with little cytoplasm and pale vesicular nucleus closely resembling the spermatogonia. The capsule is very dense and contains a few islands of tissue cells. Despite the heavy fibrous tissue the cells do not seem to be compressed. In the residual testicle tissue there is some spermatogenesis going on. This seems to represent an unusually benign, slow-growing type of the usual large cell tumor of the testicle.

Case II. A mongrel fox terrier dog, apparently moderately old, was killed in the course of experimental work in the Department of Physiology of the University of Chicago, and an autopsy was performed by Dr. A. B. Luckhardt. The right testicle, which was within the scrotum, was replaced by a tumor about 6 cm. in diameter, entirely contained within the tunica albuginea, and not adherent to the surrounding tissues. No metastases could be found in the regional nodes or elsewhere. The prostate, however, was swollen, and irregular in shape and consistence, and contained a purulent fluid. The left testicle lay within the inguinal canal and was decreased in size to about one-half the normal dimensions, although the epididymis was apparently normal. Nothing is known of the history of this animal, but it was striking in that the teats resembled those of a bitch that has nursed repeatedly.

Microscopically the tumor resembles the usual type of alveolar carcinoma seen in human cases (fig. 1). The cells are large, consisting chiefly of a large vesicular nucleus; the stroma is heavy and coarse, and divides the tumor not only into alveoli but also into lobules; only in a few areas is it cicatricial. Mitoses are abundant. A striking peculiarity in this tumor is the tendency of the cells in some alveoli to form long cords parallel to each other and at right angles to the basement membrane, producing an unusual effect of palisade arrangement.

Sections through many parts of the tumor show no residue of testicle tissue, and no teratomatous elements. The prostate shows an extensive round cell interstitial infiltration, with numerous polymorphonuclear leucocytes within the tubules. The atrophied ectopic testicle shows an epididymis resembling that of the preadolescent testicle, while the testicle itself consists of ill-defined tubules with ordinarily but a single row of irregular cells near a thickened basement membrane, although the center of the tubules contains much protoplasmic debris which is

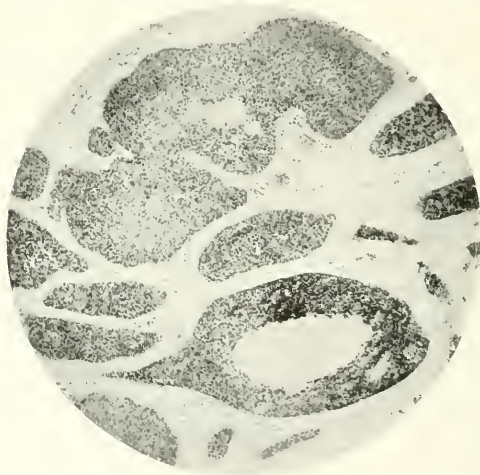


FIG. 1. PRIMARY CARCINOMA OF THE TESTICLE OF A DOG. ($\times 80$)

often pigmented. The stroma is moderately increased, but Leydig's cells are not conspicuous. Several of the tubules are full of large deeply staining cells resembling those of the tumor of the opposite testicle. These give the impression that in these isolated atrophic tubules neoplastic proliferation is beginning to arise independently.

In other species of animals, although castration is not commonly practiced, testicle tumors are rare. The reviews of the literature on spontaneous tumors of birds and fowls by Joest

and Ernesti (11), and by Pentimalli (12), mention only three testicular tumors. One was a teratoma replacing the testicle of a chicken, described by Winokuroff (13) and the other two were cases of sarcoma of the testicle in parakeets (*Palaenoris Eupatrius?*), reported by Fox (14). One of these was bilaterally symmetrical, probably derived from the testicle, and described as a large round cell alveolar sarcoma; the other was unilateral, and undoubtedly arose in the testicle. In his extensive autopsy experience with wild animals Fox has found no case of tumor of the testicle, nor could we find any such cases in the literature, unless we include the instance recorded by Pick and Poll (15) of a cystoma testis with carcinomatous areas and a secondary nodule, which occurred in the testicle of a Japanese giant salamander (*Kryptobranchus japonicus*).

From such descriptions of the histological findings of the testicular tumors of lower animals as are found in the literature, and which are mostly meagre, these growths seem to present the same characteristics as those observed in man. The prevailing type is those tumors of large polyhedral cells in alveolar arrangement, which are often described as alveolar sarcoma or, when the normal relation of the tumor cells to the nutritive vessels is conspicuous, angiosarcoma. Lymphoid infiltration of the stroma is also commonly described in animals as well as in human tumors. Ewing (16), in his critical review of testicular neoplasms, says of the human material: "The commonest tumor of the testis is an embryonal carcinoma, alveolar or diffuse, with polyhedral or rounded cells and often with lymphoid stroma." This statement holds equally well for the testicular growths of animals as described in the literature. Ewing also states: "These tumors are probably one sided developments of teratomata," an origin which is not suggested by those authors who describe animal tumors, although Caspar (4) quotes Kitt as saying that chondromas are found repeatedly in the testes of horses; on the other hand, Kimura does not even mention the occurrence in horses of tumors suggesting a teratomatous character. Furthermore, Frank (17), in his discussion of the histogenetic origin of testicle tumors, concludes that the typical

large cell tumors are not derived from teratoid tumors, but from spermatogonia. Kimura, in his study of equine material, also came to the conclusion that these tumors are derived from the epithelium of the seminiferous tubules.

TESTICULAR TUMORS IN MICE

In 19,000 autopsies on mice of the Slye stock we have found 28 tumors arising in the testicle. These figures, it should be emphasized, apply to mice living a natural life and reaching as great an age as it has been possible to make them attain by the best of care and the most rigorous hygienic precautions; all died natural deaths, without having undergone any experimental interventions or manipulation. Of the 19,000 about one-half were males. Statistics have not yet been compiled as to the total number of tumors arising in male mice, although they are much less common than in females, most abundant being tumors of the lung, subcutaneous tumors, sarcomas, tumors of the testis, and adenoma of the liver. As we have pointed out in previous papers, the great predominance of females among tumor mice, observed and emphasized by other writers, depends solely upon the frequency of mammary tumors. Excluding these and other sex gland tumors, we find an approximately equal distribution. Thus, in our published lung tumor series (18) of 160 cases there were 42.6 per cent in males, and 57.4 per cent in females; in 28 liver tumors (19) there were 50 per cent in each sex; in 8 cases of cancer of the stomach (20) also, equal numbers were observed in each sex. Sarcomas are more abundant in female mice than in males in the ratio of 2 to 1 because the mammary gland is a common site of sarcomas (21). The tumors of the testicle offset in large measure the tumors arising in the ovary.

That we have obtained so large a number of tumors of the testicle is explained by the fact that all these tumors, with one exception (7308), occurred in the mice in a single strain, No. 90, and its hybrid derivatives. This exceptional case was not a typical testicular growth, but a spindle cell sarcoma. As has

been emphasized in previous papers, not only the tendency to develop cancer depends on the ancestry of the mice, but also the localization of tumors in special organs or tissues (see Slye (22)). Had not this particular strain been developed, hybridized extensively, and followed for several years, there would have been but a single case of testicle tumor to record, and that a sarcoma. We may mention the fact that some forms of growth observed in other laboratories seem to be relatively infrequent in the Slye stock, e.g., the keratinizing type of lung tumors described by Tyzzer and Haaland, and the preputial gland adenomas observed especially in Bashford's laboratory. As material accumulates this tendency of certain tumors to occur in certain strains of mice becomes more and more distinct; this has its counterpart in human pathology—e.g., the uterine fibroids of the negro, and the repeatedly described predominance of tumors of certain viscera in certain families.

Most of these new growths of the testis are essentially benign in character, developing very slowly, rarely ulcerating, generally distinctly limited by the tunica albuginea, and in no case with distinct remote metastases (fig. 2). Some cases have remained under observation for as long as eight months, and in only a few instances have they seemed to be the cause of death, through urinary retention or ascending suppurative nephritis (5 cases), local necrosis and suppuration (3 cases), or hemorrhage (3 cases). They have varied in size from about 5 mm. in diameter to one 30 by 23 by 23 mm., the larger tumors usually showing much necrosis, although not often infected because of the protection afforded by the intact tunica albuginea. Contrary to the experience with other species, no case has been observed in an ectopic testicle, a malformation that seems to be rare in mice. As with other tumors, these growths do not appear until the mice are of middle age or older; the youngest animal in this series was 9 months old when the tumor was first seen and died when 14 months old; the oldest was $3\frac{1}{2}$ years at death, the tumor having been under observation for eight months.

Four tumors seemed to result directly from trauma, appearing in mice while under observation because of injuries to the genital

organs received in fighting. One of these was a typical spindle cell sarcoma (3117) which arose at the site of a bitten wound of the testicle, while simultaneously a similar sarcoma arose in a



FIG. 2. PRIMARY TUMOR OF THE RIGHT TESTICLE OF A MOUSE, SHOWING THE GROSS APPEARANCE USUAL IN THESE CASES

The lower pole of the tumor is necrotic. It is entirely encapsulated and there are no metastases. Microscopically this was a typical "mesothelioma" or "orchidoblastoma" as shown in figures 3 and 5.

wound on the back. Of the other three, two were of the usual large cell type (3561 and 8145) while one (585) again was a spindle cell growth. It is, of course, impossible to tell how many of the other 24 tumors followed trauma.

Several instances of simple cyst formation, probably secondary to trauma, have not been included in this series.

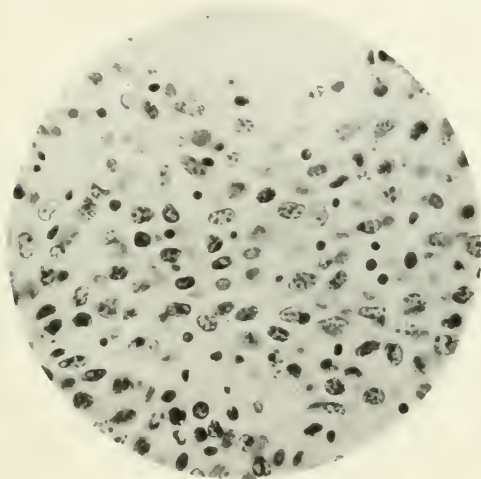


FIG. 3. SHOWING THE TYPICAL CYTOLOGICAL CHARACTERS OF MOST OF THE TUMORS OF THIS SERIES. ($\times 480$)

At the lower edge of the field is a blood-space, showing the direct relation of blood and tumor cells, without distinguishable vessel wall.

Microscopically the great majority of these tumors are all of one structure (fig. 3), which is entirely typical and characteristic. These typical growths are composed of large rather pale and delicately architected cells. The nuclei are vesicular, often markedly so, with the chromatin in coarse granules and not uniformly arranged. There is an abundant cytoplasm, staining faintly, usually with well defined margins and of polygonal

outline unless compressed into flattened or spindle shaped cells (fig. 4). Mitotic figures are fairly abundant in some specimens, but never extremely numerous; pyenosis and karyorrhexis are frequently seen. Commonly there is a tendency for the cells to be arranged in cords, as in liver tissue, and an alveolar structure is often distinct although never very well developed. As with all mouse tumors, the stroma is seldom so dense as in

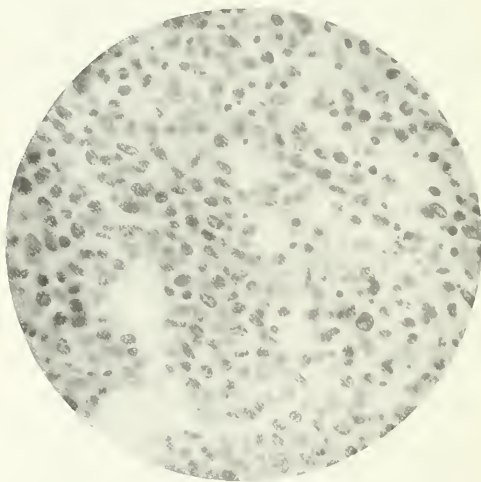


FIG. 4. TUMOR CELLS SOMEWHAT COMPRESSED INTO A MORE SPINDLE CELL TYPE. ($\times 480$)

corresponding human tumors. Usually the tumor is extremely vascular, with large blood channels (fig. 5), the walls of which are often composed solely of tumor cells, although sometimes the vessels have definite fibrous walls; hemorrhages are frequent, and in many sections old pigment and cholesterol masses indicate the site of former hemorrhages. Cells with atypical and giant nuclei are common, and multinucleated cells are often found. Sometimes tubule-like structures are present, but these cannot

be identified as typical "rosettes" such as have been described in human testicular neoplasms. Multipolar and other abnormal forms of mitosis have occasionally been seen.

Necrosis is present to greater or less degree in most of the specimens, and in some nearly all the tumor is necrotic, as if strangulated by the dense capsule. Often the necrosis is uniformly distributed in those cells more remote from the blood

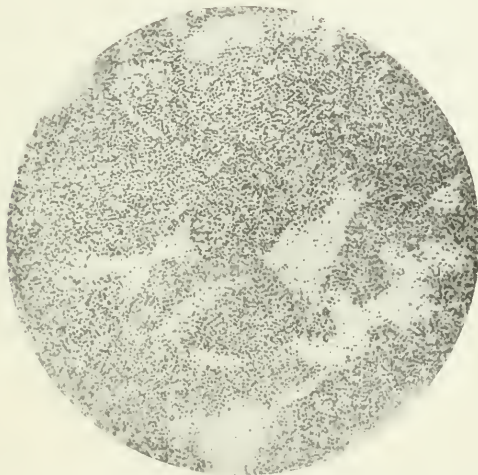


FIG. 5. SHOWING THE GENERAL HISTOLOGICAL FEATURES CHARACTERISTIC OF MOST OF THE TUMORS OF THE MOUSE TESTIS. ($\times 110$)

The intimate relation of tumor cells to blood-spaces is a striking feature.

spaces, leaving about each vessel a zone of living tumor cells so that the resulting picture resembles a "hemangiosarcoma," often quite characteristically (fig. 6). This recalls the fact that a common diagnosis of testicular tumors in man is "angiosarcoma."

In every case the original testicle tissue has been almost or quite completely destroyed, so that only occasionally can the

remains of a single isolated seminiferous tubule be found. Most often the tubules are only indicated by a partly calcified tubular outline, identified solely by the sperm heads which persist in the lumen. If more tubules remain they are generally compressed against the capsule, often heavily pigmented. The capsule of the testicle always persists, and although usually invaded, it is perforated only in a few instances; occasionally the capsule

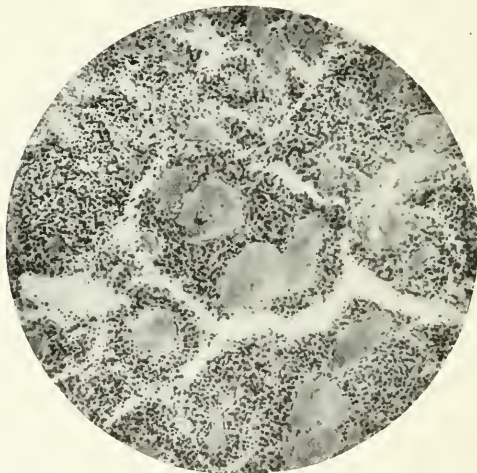


FIG. 6. TESTICULAR TUMOR IN WHICH THE TUMOR CELLS ARE GENERALLY GROUPED ABOUT BLOOD-SPACES, THUS RESEMBLING "ANGIOSARCOMA." ($\times 110$)

contains what seem to be lymph-vessels filled with plugs of tumor cells. When seminiferous tubules remain for comparison, it is usually apparent that the typical tumor cells have much the same structure as some of the cells of the tubule, the spermatogonia.

The stroma is usually scanty and very delicate, so that large areas often show no evidence whatever of stroma elements.

When best defined it divides the tumor cells into indistinct alveoli, and frequently shows an abundance of small lymphoid cells. In many instances clear cyst-like spaces are found without any distinct lining, but giving the impression of resulting from softening and absorption of the tumor, rather than from cyst formation. Blood pigment is often found in phagocytic cells in the stroma and capsule.

Five of the tumors present a histological structure resembling sarcoma, but in three of these, at least, it is probable that the sarcomatous appearance is merely the result of flattening of the cells by pressure. This assumption is supported by the finding, in some of the sarcoma-like tumors, of cell areas resembling the more usual type of testicle tumors, and by the occasional presence, in the ordinary tumors, of areas of spindle cells that are undoubtedly the result of local pressure, e.g., beneath the capsule. These tumors would seem to correspond to the type of tumor often found in the human testicle and commonly diagnosed as alveolar sarcoma or angiosarcoma. One of them (10062), however, presents so much finely divided intracellular golden pigment, without evidence of old hemorrhage as a source of this pigment, that it may possibly represent a tumor derived from the interstitial cells of Leydig, for tumors of such origin have been described in man.

Of the remaining two, one is a typical spindle cell sarcoma which has been described in our paper on sarcoma in mice (case 3117). This mouse was bitten on the back and on the genitals. From each of these sites arose a typical spindle cell sarcoma. The genital tumor seemed to arise from and replace the testicle. Both neoplasms developed at the same time, and seemed to be independent, primary, spontaneous tumors. This mouse also had an adenoma of the liver. The fifth tumor (fig. 7) also seems to be a typical spindle cell sarcoma (7308), in which no persisting elements of testicle can be found; at one point it has grown through the capsule, so that the growth is bilobed. Mallory's connective tissue stain supports the interpretation of the growth as a true sarcoma, the individual cells lying in and evidently producing a matrix of collagenous fibers.

This tumor is especially interesting as being the only growth occurring in an animal not derived from strain 90.

It is a matter for comment that we have found no example of the teratoid type of tumor that is so common in the human testicle. In not a single section have we found a trace of heterologous tissue elements that might suggest a teratomatous origin. Twice we have found simple cysts, apparently resulting

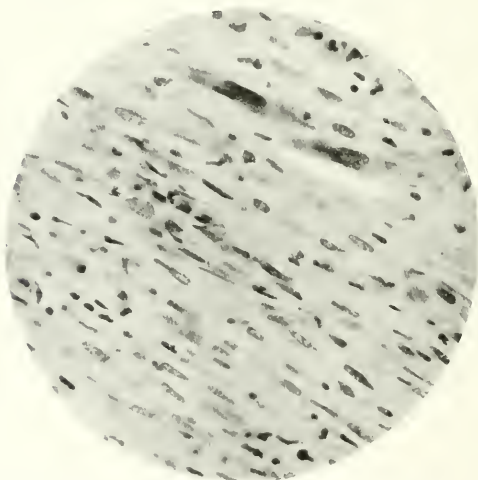


FIG. 7. SPINDLE CELL SARCOMA ARISING WITHIN THE TESTICLE OF A MOUSE. ($\times 480$)

from traumatism and subsequent absorption of the testicle elements. Indeed, in these 19,000 autopsies which have furnished so rich a tumor material, we have found but a single instance of true teratoma, and this arose in the ovary. We have made complete serial sections of three of these testicular tumors, without finding any teratomatous elements. This evidence would seem to us to indicate that, at least in the mouse, the orchidoblastomas do not ordinarily arise in teratomas, which have been

maintained by some to be the common origin of the similar tumors in man. The histological evidence of the similarity of the tumor cells to the spermatogonia would seem to support Frank's view that these neoplasms arise from the epithelium of the seminiferous tubules.

In view of the extremely heterotypical structure of these growths, the total absence of metastases in this series is surprising, especially as metastasis is so extremely extensive and early in the structurally similar tumors of man. And in other mammals, also, metastases are described from testicular tumors, especially in the horse.

One case alone (9441) showed involvement of both testicles, the structure of each growth being the same. In our first canine case, however, the animal showed no recurrence three years after orchidectomy, and the second had developed no visible metastases when autopsied. Several of our sections of mouse tumors show what appear to be plugs of tumor cells in the lymph-channels, while the blood-spaces are commonly lined with naked tumor cells, apparently offering every opportunity for extensive metastasis. Nevertheless, close inspection of the lung sections has failed to show even the retrogressing tumor cell emboli which are so often found in the lung in connection with abdominal carcinoma, as pointed out by M. B. Schmidt.

In but five cases was the tunica albuginea definitely perforated by macroscopic growths. One of these, however, showed very extensive formation of secondary nodules (7870), there being several large tumors outside the testicle (fig. 8). Here the growth consisted of six contiguous nodules, each distinct and enclosed in a capsule. Histologically all these nodules are of similar structure, characterized by an unusually large amount of lipid material and many necrotic areas. So far as structure is concerned this tumor does not seem to be more malignant than other tumors that do not show the multiple nodules. In one case (2753) the growth extended along the spermatic cord, so that the mass resembled a hernia.

As noted in previous studies of special types of tumors in mice, animals with one tumor are likely to exhibit a growth of

some other sort, apparently more often than would be explained by the law of probability. With testicular tumors the co-existence of other neoplasms would not be expected to be so

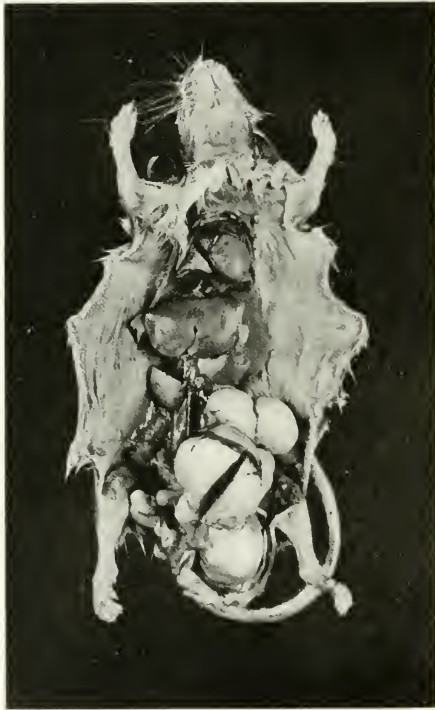


FIG. 8. TUMOR OF THE LEFT TESTICLE WITH THE PRODUCTION OF SEVERAL LOCAL METASTATIC GROWTHS, BUT NO REMOTE METASTASIS

frequent as was observed with the tumors previously described (lung tumors, liver tumors, sarcomas) because the commonest of all tumors in mice, the mammary carcinoma, does not often

occur in males. Nevertheless, among our 28 cases of tumor of the testis we find the following seven instances of multiple tumor formation: One mouse with a subcutaneous spindle cell sarcoma and an adenoma of the liver; three with papillary adenomatous growths arising in the lung; two with osteosarcomas, one arising in the thigh (16370), the other in the subcutaneous tissues near the fore leg (8745). In still another mouse there was a subcutaneous growth in the neck of such a character that a diagnosis has not been made (5037). This growth was about 1 cm. in diameter and 2 to 3 mm. thick, consisting of a mass of cells with strongly basophilic cytoplasm, of a finely granular, "ground glass" character, resembling that of plasma cells. The nuclei, however, are centrally located and have not the characteristic chromatin arrangement of the plasma cell nucleus. The cell boundaries are distinct, and the cells tend to form long rows and strings, recalling the structures sometimes seen in the so-called "lymphangioma hypertrophicum." It is probable that this growth is a true neoplasm, but as long as its nature remains undecided its neoplastic character cannot be definitely determined. The character of the cells is such as to make it improbable that it is a metastatic growth. The occurrence of multiple primary new growths in no less than 25 per cent of these male tumor mice is striking evidence of the existence of a predisposition to tumor formation.

SARCOMA OF THE SEMINAL VESICLE

In the literature on tumors of the lower mammals we have found mention of but one instance of a neoplasm arising in the seminal vesicles. Such growths are also rare in man, C'eelen (23) in 1912 having been able to find reports of but five cases of carcinoma and one of sarcoma, to which he added a case of fibromyoma. We have not been able to find records of any other human or animal cases, except that described by Flexner and Jobling (24) as "originally regarded as a sarcoma; probably a teratoma, from which an adeno-carcinoma was developed." This tumor, which has been used extensively in transplantation,

was found in a white rat, as a tumor "the size of a walnut" attached to the left seminal vesicle, without production of metastases. It was first described as a polymorphous sarcoma, but with included glandular elements. In the course of transplantation the carcinomatous elements developed and replaced the sarcomatous structures, becoming established as an adeno-carcinoma by the twenty-eighth generation.

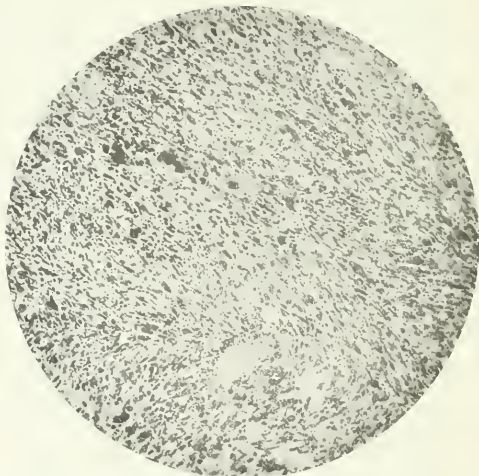


FIG. 9. PRIMARY SARCOMA OF THE SEMINAL VESICLE OF A MOUSE. ($\times 110$)

Note the numerous giant cells, and the polymorphous character of the tumor cells.

A single case of primary tumor of the seminal vesicle has been observed in one of the mice examined in this laboratory (15443). This animal died of pneumonia, and autopsy disclosed the left seminal vesicle constricted at a point midway of its length, with the tip shrunken. The remainder of the organ is distended into a mass 10 by 8 by 8 mm., rather soft, but containing no fluid. The right seminal vesicle is similarly con-

stricted, but there is no tumor tissue and the tip is merely distended with yellow secretion; the rest is in shape like the left, but slightly smaller and yellow in color. There were no metastatic growths to be found. The testicles seem normal, and the kidneys show a slight nephritis. Microscopically the lumen of the right vesicle is found to be distended by a solid cellular growth, limited by the somewhat thickened peritoneal coat and the remains of the muscular coat, except at one point where these structures have been penetrated by the neoplasm. Only a few small groups of columnar epithelial cells remain to represent the original mucosa. The tumor is composed of spindle cells of varying size, but mostly larger than in the usual spindle cell sarcoma (fig. 9). There are also many multinucleated cells, and numerous enormous single cells with a single giant nucleus, sometimes undergoing asymmetric direct division. Thin-walled blood-channels and blood-spaces lined only by unmodified tumor cells are abundant. There are no hemorrhages or necrotic areas, but some cystic spaces full of plasma. This is a typical large polymorphous celled sarcoma. Nothing was found to indicate a teratomatous or carcinomatous character, such as was observed in the Flexner-Jobling rat tumor, yet the fact that theirs was at first diagnosed as a polymorphous cell sarcoma is suggestive. In our case no transplantation experiments were attempted.

SUMMARY

Among 19,000 mice dying natural deaths and examined post mortem, about one-half of which were males, 28 instances of primary tumor of the testicle were found. Most of these resembled in all essential features the tumors that arise in the testicle of man and other animals, consisting of cells closely resembling the epithelium of the seminiferous tubules, arranged in an alveolar structure. Despite great vascularity and a markedly atypical structure, no remote metastasis was observed, although in one case a series of six contiguous independent nodules was formed, and one case showed bilateral testicular tumors. Two of the growths seemed to be true spindle cell sarcomas, one arising at the site of a wound. Three of the typical "or-

chidoblastomas" also followed trauma. No evidence could be obtained that any of these tumors had arisen in a teratomatous growth, and no cases of teratoma have been observed.

One case of polymorphous cell sarcoma of the seminal vesicle of a mouse is described, apparently the second case of a tumor of this organ reported in a lower animal.

Two cases of primary spontaneous tumor of the testicle in dogs are described.

With the exception of one sarcoma, all the 28 neoplasms of the mouse testis occurred in the members of a single strain of mice and its hybrid derivatives, thus substantiating the statement that heredity influences the incidence of tumor development in different organs or tissues. This fact also probably explains the absence of any recorded cases of tumor of the testis in mice from other laboratories.

REFERENCES

- (1) MCCOY: Jour. Med. Research, 1909, N.S., xvi, 285.
- (2) WOOLLEY AND WHERRY: Jour. Med. Research, 1911, N.S., xx, 205.
- (3) BEATTI: *Semana med.*, 1917, xxiv, 643.
- (4) CASPAR: Lubarsch-Ostertag, *Ergebn. d. allg. Path.*, 1896, iii², 799.
- (5) KIMURA: *Gann*, 1917, xi, 38.
- (6) STICKER: *Arch. f. klin. Chir. (Langenbeck)*, 1902, lxv, 616.
- (7) TROTTER: Jour. Comp. Path. and Therap., 1911, xxiv, 1.
- (8) MURRAY: Third Sci. Report, Imperial Cancer Research Fund, London, 1908, p. 41.
- (9) WILLIAMS: *The Natural History of Cancer*, p. 96.
- (10) WOLFF: *Die Lehre v. d. Krebskrankh.*, 1913, iii¹, 217.
- (11) JOEST AND ERNESTI: *Ztschr. f. Krebsforsch.*, 1915, xv, 1.
- (12) PENTIMALLI: *Ztschr. f. Krebsforsch.*, 1915, xv, 111.
- (13) WINOKUROFF: *Inaug. Diss.*, Bonn, 1908; cited by Joest and Ernesti.
- (14) FOX: Jour. Path. and Bacteriol., 1912, xvii, 217.
- (15) PICK AND POLL: *Berl. klin. Wehnschr.*, 1903, xl, 572.
- (16) EWING: *Surg., Gynec., and Obst.*, 1911, xii, 230.
- (17) FRANK: *Frankfurt. Ztschr. f. Path.*, 1911, ix, 206.
- (18) SLYE, HOLMES, AND WELLS: Jour. Med. Research, 1914, N.S., xxv, 417.
- (19) SLYE, HOLMES, AND WELLS: Jour. Med. Research, 1915, N.S., xxviii, 171.
- (20) SLYE, HOLMES, AND WELLS: Jour. Cancer Research, 1917, ii, 401.
- (21) SLYE, HOLMES, AND WELLS: Jour. Cancer Research, 1917, ii, 1.
- (22) SLYE: Jour. Cancer Research, 1916, i, 479.
- (23) CEELLEN: *Virchows Arch.*, 1912, ccvii, 200.
- (24) FLEXNER AND JOBLING: *Monographs of the Rockefeller Institute*, 1910, No. 1; Jour. Amer. Med. Assn., 1907, xlviii, 420; *Centralbl. f. allg. Path. u. path. Anat.*, 1907, xviii, 257.

CANCER IN HAINAN, CHINA

A PRELIMINARY STATISTICAL STUDY OF 131 OPERATIONS WITH SPECIAL REFERENCE TO AGE INCIDENCE, ANATOMICAL DISTRIBUTION, AND ETIOLOGY

NATHANIEL BERCOVITZ

Kachek, Hainan, China

Received for publication, June 20, 1918

Cancer is regarded as a disease of the later decades of life. Its etiology is still obscure, although many contributing causes such as continued local irritation of a chemical, mechanical, or inflammatory nature have been held responsible. Indeed, an almost innumerable list of possible factors, including bacteria and protozoa, has been studied with reference to etiology, but cancer still remains withal a problem unsolved.

The disease, as observed by the author in Hainan, presents a number of phases which differ somewhat from those usually encountered. These phases include the early age incidence of the disease, the unusual anatomical distribution, and a difference in the causes commonly considered as predisposing.

In the study of these statistics the medical conditions peculiar to China must be remembered, viz., the gradual turning of the people to western medicine, the fact that many cases which come to the hospital are inoperable and come only after all native remedies have failed, and, finally, the fact that until very recently prejudice has kept the women of China away from medical and surgical aid. For these reasons it may be assumed that the statistics of the next few years will differ somewhat from the present figures.

The cases were operated upon by the author in Hoi How and Kachek, and by members of the staff in the Hoi How Hospital. Lack of facilities prevented microscopic examination, to the great regret of the writer.

AGE INCIDENCE

Table 1 shows the proportion of cases seen in the early decades of life. This fact is mentioned without comment, except to say that the percentage is unusually high.

TABLE 1

Total number of cases, 131

AGE	NUMBER OF CASES	PER CENT
Above 40 years.....	65	49.7
Under 40 years.....	66	50.3
	131	100.0
Under 35 years.....	47	36.0
Under 30 years.....	38	29.0
Under 25 years.....	25	19.0

There are no known etiological factors which would account for 50.3 per cent of the cases occurring before the age of forty.

ANATOMICAL DISTRIBUTION

Table 2 groups the cases roughly according to anatomical location, with reference to the age incidence and sex.

TABLE 2

ANATOMICAL DISTRIBUTION	NUMBER OF CASES	PER CENT	AGE		MALE	FEMALE
			Above 40	Under 40		
Penis.....	29	22.1	18	11		
Skin.....	29	22.1	16	13	23	6
Neck.....	26	19.8	8	18	22	4
Face; Scalp.....	21	16.0	9	12	17	4
Breast.....	10	7.6	7	3	2	8
Bone.....	8	6.1	3	5	6	2
Testicle.....	3	2.4	2	1		
Anus.....	3	2.4	1	2	2	1
Tongue.....	1	0.7	1	0	1	0
Colon.....	1	0.7	0	1	1	0

The most noticeable feature of table 2 is the high percentage of carcinoma of the penis. Except for the high incidence of syphilis among the Chinese male population and attempts to cure the primary sore by the application of highly caustic native remedies, no reason can be assigned for this large figure.

Under the heading of "skin" are grouped all the cancers of the skin covering the extremities and the trunk. These are often encountered, perhaps because ulcers of the arms and legs are very common in China. The ulcers are treated, not by cleansing and the application of healing remedies, but by the crude native methods of applying plasters, either of mud mixed with cow dung, or of leaves enclosing irritating substances. As a result, they eat deeply into the flesh and heal—if they heal at all—with contractures of the muscle sheaths and consequent deformity; or they produce an exuberance of scar tissue with the formation of keloids; or they degenerate into malignant growths.

Counter irritation is extensively practiced by the Chinese with the idea of driving out the evil spirits supposed to be the cause of disease. The counter irritation takes many forms, one of which is a piercing of the skin. The resulting wound is always infected, and in healing may degenerate into a malignant growth.

Malignant disease of the cervical glands is frequently seen. The table shows that 26 of the 131 cases belong to this class, and in the experience of the author most of the inoperable cases of cancer met with in Hainan have been of the neck. The etiology in these cases is exceedingly obscure, as the great proportion of cases occur in the period under forty years of age, one case being only four years of age. In most of the cases there are no marks on the neck to indicate previous counter irritation, and infection from the throat might well be considered a contributing factor, especially in the absence of any other signs.

Cancer of the face and scalp includes epithelioma of the lip, malignant disease of the nose, and cancer of the skin of the face and scalp. A large proportion of these cases occur in the early decades of life, and aside from the considerations discussed in

cases of cancer of the skin it might be well to mention the fact that most of these people are much exposed to the rays of the tropical sun. This exposure has been regarded as a cause of rodent ulcer.

When the cases of skin cancer are grouped with those of the face and scalp, a total of 50 cases, or 38.1 per cent of the whole number reported, fall under this group; which, with carcinoma of the penis and of the glands of the neck make a total of 105 cases, or about 80 per cent of all the cases.

This leaves for consideration the following:

Cancers of the breast, 70 per cent of which are seen after the fourth decade. The number reported is only 10; but it must be remembered that the traditions of centuries have kept women from seeking aid from male physicians. In view of the changing conditions, the next few years should add greatly to our knowledge of this disease in China.

Eight cases of osteo-sarcoma present no unusual features. Three cases of carcinoma of the testicle, three of epithelioma of the anus, and one case of carcinoma of the descending colon are not unlike similar cases found elsewhere.

Two locations, so common in America and many other countries, are strikingly conspicuous by their absence from this list, viz:—the stomach and the uterus. Here again the existing conditions in China must be taken into consideration. The clinical diagnosis of cancer has occasionally been made, but not frequently, and only recently have abdominal operations of any kind been undertaken. Hence the physicians may not see the cases, especially as the Chinese like to treat such patients themselves. Or there may actually be a smaller number of cases because certain contributing factors are absent. Thus, while they do eat a little meat and a few vegetables, the diet of the Chinese is chiefly rice, and excesses in diet can not be afforded by the masses, who are very poor.

Ulcer of the stomach is rather infrequent. And as it has been shown by Rosenow and others that gastric ulcer is the result of streptococcic infection, it will be of further interest to know that scarlet fever and diphtheria are not known in Hainan, and

that appendicitis and acute articular rheumatism (non-gonorrheic) are only infrequently met with. In the absence of much gastric ulcer, therefore, the cases of cancer of the stomach might be expected to be few.

It is also of interest to note that the gastric crises of tabes dorsalis and general paresis are very rarely seen, notwithstanding the widespread incidence of syphilis and its very destructive tertiary lesions.

As to cancer of the uterus, the author has seen one case, which was inoperable. It is expected that more will be seen in the future, but it must be noted that uterine fibroids are rare; and notwithstanding the many cases of gonorrhea among men, cases of salpingitis and vaginitis seem to be very rare. Furthermore, stricture of the male urethra is not very common, and the question might very well be raised whether the gonococcus in Hainan may not be less virulent, because of the heat of the tropics. Still, this could hardly be the case, because it is as potent in its destructive influences on the eyes, the epididymis, and the joints as it is in America. On the other hand, are the cells lining the urethra, the tubes, uterus, and vagina less sensitive to the gonococcus in Hainan than in America? If inflammation of the female generative organs be considered a cause of malignancy, that cause, as far as is now known, seems to be largely lacking in Hainan.

Otherwise the lesions of the female generative organs are the same as in America, with the possible exception that malpositions are less frequent because of the more normal life and habits of the women here.

This brief statistical study of cancer in Hainan brings out the following points:

1. That cancer in Hainan is as much a disease of the early decades of life as the later, for which no reason can be assigned.
2. That cancer of the penis, and glands of the neck is unusually frequent; for the former an old primary sore may be the etiological factor; for the latter no reason can be assigned.
3. That cancer of the exposed surfaces of the body is very common. Inasmuch as in most cases these people wear a

scanty amount of clothing, the arms, trunk, and legs of men who work the fields being bare, the rays of the sun may be a contributing factor. But it is rather more likely that large ulcers, treated in the native fashion, and the sores resulting from counter irritation as practiced by the Chinese, are the etiological factors. This is all the more probable in view of the tendency to keloid formation and the universal infection of wounds.

4. Cancer of the stomach is infrequent. The absence of certain virulent streptococcic infections which are responsible for gastric ulcer may have something to do with this; or it may be that in this case, as in cancer of the uterus, the surgeon has not seen the cases.

5. Cancer of the uterus is infrequent. It may be that these cases have not been seen by the physician, but it seems rather suggestive that the virulent infections of the female generative organs seem to be infrequent here.

MORTALITY STATISTICS OF CANCER AMONG WAGE EARNERS: WITH OBSERVATIONS ON THE COMPARATIVE INCIDENCE OF THE DISEASE IN THE GENERAL POPULATION

LOUIS I. DUBLIN

Statistician, Metropolitan Life Insurance Company, New York

Received for publication, August 26, 1918

Although there have appeared in recent years many and important contributions to the mortality statistics of cancer, few of these have presented the facts in such detail as to show the incidence of this condition in the several age periods of life with the further distinction of sex and of color or race of the population. This is the merit of the data presented herewith.¹ They are to our knowledge original in the cancer literature and should well serve the nation-wide movement for the control of the disease. Many discussions which have centered around the cancer problem in recent years, such as the supposed increase of mortality and other questions, can be settled only as we know for a period of years the detailed facts of mortality for a relatively constant population. The present experience meets this requirement admirably and has the further merit that it reflects conditions in a large industrial group among whom, as will be shown later, cancer takes a heavy toll.

IMPROVEMENT OF CANCER MORTALITY STATISTICS

Special efforts were made in the course of the present inquiry to have the basic data as reliable as possible. Physicians certifying the causes of death often returned statements of cancer

¹ Being chapter ix of volume entitled: *Mortality Statistics of Insured Wage Earners and their Families in the United States and Canada*. Metropolitan Life Press, New York, 1919.

unqualified as to the organ or part affected, and in such instances, letters of inquiry were written and the physicians were asked to specify the type of tumor or cancer and the organs or parts first affected by the growth. The effect of this correspondence has been to increase the precision of the statistical results for cancer. While the data were not refined to the same point of completeness as characterized the recent investigation of the United States Bureau of the Census (1), the effort was made to cover fully the various parts and organs specified in the International List of Causes of Death.

CANCER ACCORDING TO ORGANS OR PARTS

During the six year period of this investigation, 37,666 cancer deaths were recorded at a rate of 70.0 per 100,000 persons exposed. Cancer was the sixth cause in order of numerical importance in this study. These deaths constituted 5.9 per cent of all the deaths in the experience. In the following table, the facts are arranged so as to show the number of deaths from cancer of the various organs or parts, and a few derivative ratios, including the death rates per 100,000 persons exposed are given:

TABLE 1

Mortality from cancer, specified according to organs or parts affected. Deaths, and death rates per 100,000 persons exposed. All color and sex groups combined. Experience of Metropolitan Life Insurance Company, Industrial Department, 1911 to 1916

Organ or Part Affected.	All Color and Sex Groups in Mortality Experience.			
	No. of Deaths.	Per Cent. of Total—All Causes.	Per Cent. of Total Cancer Deaths.	Death Rate per 100,000 Exposed.
CANCER—ALL FORMS.....	37,666	5.9	100.0	70.0
<i>Cancer of the:</i>				
Buccal cavity.....	1,353	.2	3.6	2.5
Stomach, liver.....	14,153	2.2	37.6	26.3
Peritoneum, intestines, rectum...	4,482	.7	11.9	8.3
Female genital organs.....	7,882	1.2	20.9	14.7
Breast.....	3,579	.6	9.5	6.7
Skin.....	938	.2	2.5	1.7
Other organs, or of organs not specified.....	5,279	.8	14.0	9.8

Cancer and other malignant tumors of the stomach and liver constituted the largest single group of malignant growths, with 37.6 per cent of all cancers, at a rate of 26.3 per 100,000 persons exposed. Cancer of the female genital organs was next in importance, with 7882 deaths, constituting 20.9 per cent of all cancer deaths with a rate of 14.7 per one hundred thousand persons of both sexes. Cancers affecting the peritoneum, intestines, and rectum followed with 4,482 deaths, in all 11.9 per cent of all cancers at a rate of 8.3 per 100,000. These death rates, however, vary considerably with age and sex. In the following table, we give a comparison of the general cancer mortality experience for each of the main color and sex classes, showing separately the facts for cancer of the various organs or parts. The age data will be presented later.

TABLE 2

Mortality from cancer, classified according to organs or parts. Percentage of deaths, all causes, and death rate per 100,000 persons exposed. By color and by sex, 1911 to 1916. Experience of Metropolitan Life Insurance Company, Industrial Department, 1911 to 1916.

Organ or Part Affected.	Total Experience.		White Males.		White Females.		Colored Males.		Colored Females.	
	P. C. of Deaths—All Causes.	Death Rate per 100,000.	P. C. of Deaths—All Causes.	Death Rate per 100,000.	P. C. of Deaths—All Causes.	Death Rate per 100,000.	P. C. of Deaths—All Causes.	Death Rate per 100,000.	P. C. of Deaths—All Causes.	Death Rate per 100,000.
CANCER—ALL FORMS.....	5.9	70.0	4.3	50.4	8.5	88.4	1.8	31.0	5.2	87.8
<i>Cancer of the:</i>										
Buccal cavity.....	.2	2.5	.4	4.6	.1	.9	.1	2.5	.1	1.3
Stomach, liver.....	2.2	26.3	2.1	24.8	2.9	29.8	.9	16.2	1.1	18.5
Peritoneum, intestines, rectum.....	.7	8.3	.6	6.6	1.0	10.5	.2	3.4	.4	7.2
Female genital organs...	1.2	14.7	—	—	2.4	25.3	—	—	2.2	37.9
Breast.....	.6	6.7	—	.1	1.1	11.7	*	.3	.9	14.7
Skin.....	.2	1.7	.2	2.2	.2	1.6	*	.8	*	.7
Other organs or of organs not specified.....	.8	9.8	1.0	12.0	.8	8.6	.4	7.9	.4	7.3

*Less than .05 per cent.

STATISTICS ACCORDING TO COLOR, SEX, AND AGE

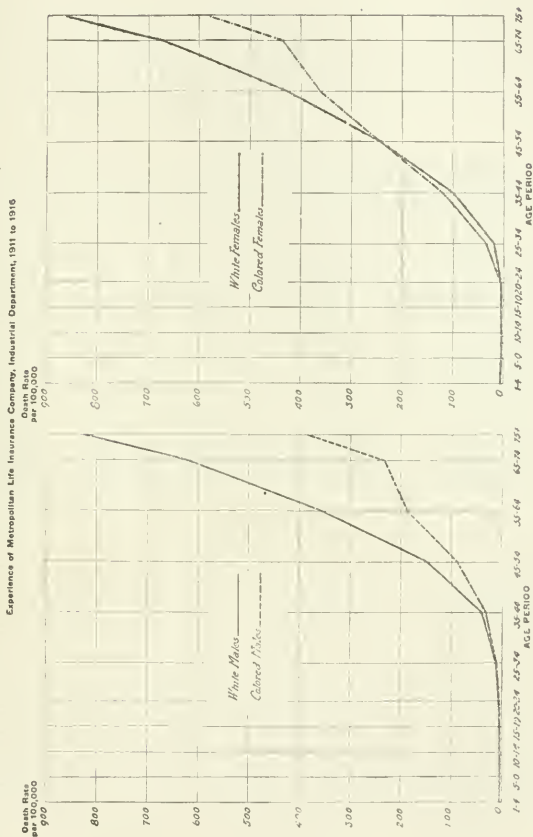
We see from this table that for all ages, one and over combined, white persons show higher cancer death rates than colored persons, although the white female rate is only slightly higher

than that for colored females. Various differences between the cancer death rates of the color and sex classes occur for this disease as it affects various organs or parts. White males, for instance, show uniformly higher cancer death rates for each of the organs or parts, when compared with the rates for colored males. White females show significantly lower cancer death rates for this disease only as it affects the female genital organs and the breast. Cancer of the uterus and of the other genital organs shows a rate of 25.3 per one hundred thousand white females exposed and a rate of 37.9 for colored females. Cancer of the breast, in this present mortality experience, was recorded at a rate of 11.7 per one hundred thousand white females, and at a rate of 14.7 per one hundred thousand colored females. For the other chief organs or parts, cancer mortality of white females is greater than among colored females. In a later part of this present section, we shall bring out in greater detail the age characteristics of this cancer mortality experience for the several color and sex classes, and with distinction of the several organs and parts. We quote below our general cancer death rates per one hundred thousand persons exposed in each of the color and sex classes for the various age periods. The accompanying chart shows the course of the cancer death rates in the experience according to age, for each of the color and sex classes.

In the introduction to this present section, we indicated that, in general, cancer mortality was lower among colored persons than among white persons, and that colored males showed relatively more favorable rates than colored females. Without any emphasis at this present time upon the organs or parts affected by cancer, we may now consider the general age characteristics of this disease.

A fairly significant rate is registered for the ages one to four years in the total experience. The cancer rate declines thereafter to its minimum at ten to fourteen years of age and then rises, at first gradually, but afterward in heavy increments up to the latest age period in this series. The same general characteristics of the age course of cancer mortality are observed for white males as for white females, with the exception that the

Death Rates per 100,000 Persons Exposed, Classified by Age Periods. White and Colored Groups Compared for Each Sex Class



upward slope of the curve for white females is very much sharper for the ages beyond 25 years. The cancer death rates for colored persons under 25 years of age are, for the most part, very low, and fluctuate somewhat irregularly. Beginning with the age period 25 to 34 years, however, there is a constantly increasing rate, up to the highest age period recorded in this series.

TABLE 3

Mortality from Cancer, all forms, classified by color, by sex, and by age period. Death rates per 100,000 persons exposed, 1911 to 1916. Experience of Metropolitan Life Insurance Company, Industrial Department, 1911 to 1916

Age Period.	Persons.	White.		Colored.	
		Males.	Females.	Males.	Females.
All ages—one and over ..	70.0	50.4	88.4	31.0	87.8
1 to 4.....	3.7	3.7	4.0	2.6	2.5
5 to 9.....	1.4	1.5	1.6	1.0	1.0
10 to 14.....	1.3	1.5	1.4	.3	.6
15 to 19.....	2.8	2.8	2.8	2.2	2.9
20 to 24.....	4.1	4.6	3.7	1.3	6.3
25 to 34.....	15.7	8.8	18.5	7.5	33.2
35 to 44.....	76.2	38.3	99.3	30.0	118.1
45 to 54.....	198.6	147.0	238.6	84.7	238.7
55 to 64.....	382.5	356.2	423.2	183.1	359.1
65 to 74.....	617.2	625.3	665.1	230.5	433.4
75 and over.....	818.2	822.8	863.9	384.1	580.7

COLOR RATIO OF CANCER MORTALITY

White males show emphatically higher cancer death rates at every age period than were recorded for colored males. Comparisons between the cancer death rates of white and colored females are practicable beginning with the age period 25 to 34 years. Between 25 and 44 years, the cancer death rate of white females was decidedly lower than the rate for colored females. Between 45 and 54 years, the rates were practically the same. Beginning with the age period 55 to 64 years and continuing to the highest age period in the table, we observe that the cancer death rates of white females were much higher than the rates for colored females. These differences in the total cancer death rates of white and colored females are to be accounted for, as will be shown later, by the higher mortality from cancer of the generative organs among colored females.

SEX RATIO OF CANCER MORTALITY

We have seen that among white lives the cancer death rate of females was practically one and two-thirds that of males. Cancer mortality of white males exceeds that of white females only for cancer of the buccal cavity, where the rates are 4.6 and .9 per 100,000 persons exposed, respectively; for cancer of the skin, where the rates are 2.2 and 1.6 respectively, and for the group of "cancers of other organs or of organs not specified." For cancer of the stomach and liver and of the peritoneum, intestines, and rectum, the death rates of white females were decidedly in excess of those for white males. In addition, white females showed a high death rate for cancer of the female genital organs (25.3 per one hundred thousand) and for cancer of the breast (11.7 per one hundred thousand). Practically the same general remarks apply to the comparative cancer death rates of colored males and colored females when compared with respect to the several organs affected by malignant growths.

There are no important differences in the cancer mortality of the two sexes among white lives under 25 years of age. Beginning with the age period 25 to 34 years, however, the cancer death rates of white females exceed those of white males substantially, up to and including the age period 55 to 64 years. Thus at the age period 35 to 44 years the rate for white males was only 38.6 per cent of that for white females. After age 65, the disproportion between the rates for the two sexes among white lives is not so great.

The excess of the cancer death rate of colored females over the rate for colored males is much greater than was observed, age period by age period, for white lives. Thus between 25 and 45, the rate for colored males was only about one-fourth as great as for colored females. In the following table, we give, first, a statement of the ratio of cancer mortality between the two races and, second, the sex ratio according to age period:

TABLE 4

Mortality from cancer (all forms). Percentages: Colored of White Mortality by Sex; Male of Female Mortality by Color; Classified by Age Period. 1911 to 1916. Experience of Metropolitan Life Insurance Company, Industrial Department, 1911 to 1916.

Age Period.	Per Cent. Colored of White Mortality.		Per Cent. Male of Female Mortality.	
	Males.	Females.	White.	Colored.
ALL AGES—ONE AND OVER..	61.5	99.3	57.0	35.3
1 to 4.....	70.3	62.5	92.5	*
5 to 9.....	66.7	62.5	93.8	*
10 to 14.....	20.0	42.9	107.1	*
15 to 19.....	78.6	103.6	100.0	*
20 to 24.....	28.3	170.3	124.3	*
AGES 25 AND OVER.....	43.0	80.1	70.5	37.9
25 to 34.....	85.2	179.5	47.6	22.6
35 to 44.....	78.3	118.9	38.6	25.4
45 to 54.....	57.6	100.0	61.6	35.5
55 to 64.....	51.4	84.9	84.2	51.0
65 to 74.....	36.9	65.2	94.2	53.2
75 and over.....	46.7	67.2	95.2	66.1

*Insufficient data.

COMPARISON OF CANCER DEATH RATES AMONG INSURED WAGE
EARNERS WITH RATES FOR POPULATION OF EXPANDING
REGISTRATION AREA OF THE UNITED STATES

For both males and females, at all ages one and over combined, the cancer death rates of white lives in the insurance experience are substantially lower than the rates recorded in the Registration Area of the United States. This favorable ratio for the cancer mortality experience of insured wage earners does not hold for all of the age periods. Thus, the cancer death rate among white male wage earners is, in general, lower than the rate for males in the general population only at ages under 35 years. Among white insured females, the cancer death rate is lower than the rate among females in the general population only between 20 and 35 years of age.

The cancer death rate among both white males and white females of the insurance experience was higher than the rate for males and females in the Registration Areas at these age periods,

beyond 35 years of age, where cancer is of the most importance as a cause of death. It should be remarked also that male wage earners show much greater percentages of excess in cancer mortality than do females in wage earners' families. The following table sets forth these comparative facts of cancer mortality:

TABLE 5

Mortality from cancer (all forms). Death rates per 100,000 persons exposed. Classified by sex and by age period. Insured white lives in experience of Metropolitan Life Insurance Company, Industrial Department (1911 to 1916) and general population experience of expanding registration area of the United States (1910 to 1915).

Age Period.	Males.			Females.		
	M. L. I. Co. (White).	U. S. Reg. Area.	Per Cent. M. L. I. Co. of Reg. Area.	M. L. I. Co. (White).	U. S. Reg. Area.	Per Cent. M. L. I. Co. of Reg. Area.
All ages—one and over	50.4	62.2	81.0	88.4	97.9	90.3
1 to 4.....	3.7	3.6	102.8	4.0	3.1	129.0
5 to 9.....	1.5	2.0	75.0	1.6	1.4	114.3
10 to 14.....	1.5	1.7	88.2	1.4	1.4	100.0
15 to 19.....	2.8	2.9	96.6	2.8	2.6	107.7
20 to 24.....	4.6	4.4	104.5	3.7	4.6	80.4
25 to 34.....	8.8	9.3	94.6	18.5	20.8	88.9
35 to 44.....	38.3	31.9	120.1	99.3	89.0	111.6
45 to 54.....	147.0	109.8	133.9	238.6	227.0	105.1
55 to 64.....	356.2	280.3	127.1	423.2	406.9	104.0
65 to 74.....	625.3	503.4	124.2	665.1	607.0	109.6
75 and over.....	822.8	710.2	115.9	863.9	828.2	104.3

POSSIBLE RELATION OF CANCER TO ECONOMIC CONDITION OR SOCIAL STATUS

At this point in the discussion, brief reference may be made to the possible relation between the incidence of cancer mortality and economic status, as indicated in a paper recently published on this subject (2). The following table shows the main facts of an investigation based upon the comparative mortality experience of the Ordinary, Intermediate, and Industrial Departments of the Metropolitan Life Insurance Company during the three years 1914, 1915, and 1916. White lives only were included in this investigation. The Ordinary Department policyholders are drawn from higher economic strata of the population

than are the Intermediate group. The Industrial policyholders form the third class or group in order of material circumstance. In order to eliminate the slight effect of medical selection in the Ordinary and Intermediate groups with respect to cancer, we considered only the mortality in these classes on business in force at least five years.

TABLE 6

Cancer claim rates per hundred thousand mean in force. Ordinary and Intermediate departments, first five years of issue excluded, compared with Industrial Department, all years of issue combined. Composite mortality experience 1914, 1915, and 1916. White lives. By sex and by age period.

Sex and Age Period.	Ordinary Department.	Intermediate Branch.	Industrial Department.
MALES:			
Ages 25 and over	83.5	70.3	140.0
25 to 34	12.0	8.7	9.7
35 to 44	33.4	41.8	37.5
45 to 54	104.3	107.6	154.1
55 to 64	276.5	295.1	368.0
65 and over	662.5	645.3	679.2
FEMALES:			
Ages 25 and over	141.6	115.1	197.7
25 to 34	31.4	25.4	17.8
35 to 44	71.6	87.8	98.9
45 to 54	213.5	206.7	235.8
55 to 64	353.6	422.1	429.6
65 and over	313.1	1,009.8	707.5

In this table, *claim rates* per one hundred thousand mean in force for the several departments are compared. Actual experience demonstrates that very little error is involved in a comparison of mortality rates based upon the number of claims reported and the mean number of policies in-force if such data are related strictly to age periods. The comparison is valid therefore as above given. Because of the heavy representation of policyholders at the ages under 45 years, with a small number of deaths, leading to aberrant cancer death rates for these ages, the Intermediate rates for both males and females at all ages are apparently the lowest. At the ages beyond 45 years, where cancer mortality is numerically significant, the Industrial group showed the highest rate, the Ordinary the least, and the Intermediate a

rate between the other two. As a result of an extended consideration of the data developed in this inquiry into the possible relation of cancer and economic condition, it was concluded that:

1. The current medical opinion that there is a strong association between low economic status and a low cancer death rate is in all probability unfounded.

2. The cancer mortality rate at the ages where the cancer rate is significant decreases as we go up in the economic scale.

3. This is true for both sexes and by age period where sufficient data are available.

4. This conclusion is not conditioned by the effect of varying amounts of medical selection in the three groups considered.

TREND OF THE CANCER DEATH RATE

Medical literature of the past few years contains much controversy on the question whether mortality from cancer is actively increasing or not. One school of research holds "that the mortality from cancer is increasing at a more or less alarming rate throughout the entire civilized world and that this increase implies most serious consequences, present and future, to the populations concerned" (3). Another group of statisticians holds that "the reported mortality from cancer is increasing in almost every part of the world, but the real mortality, if increasing at all, is certainly not increasing with equal rapidity. . . . The cumulative evidence that improvements in diagnosis and changes in age composition explain away more than half, and perhaps all of the apparent increase in cancer mortality, rebuts the presumption raised by the figures and makes it probable, though far from certain, that cancer mortality is not increasing" (4).

It is not the purpose of this section to take sides in the controversy. It is desired to offer the mortality records of the present investigation only as a contribution to the available supply of trustworthy data on the trend of cancer mortality. The following table shows the rates per 100,000 persons exposed in each of the color and sex classes within the scope of this inquiry for the years 1911 to 1916:

TABLE 7

Mortality from cancer, all forms, classified by color and by sex. Death rates per 100,000 persons exposed. Single years in period 1911 to 1916. Experience of Metropolitan Life Insurance Company, Industrial Department.

Year.	Persons.	White.		Colored.	
		Males.	Females.	Males.	Females.
1911 to 1916.....	70.0	50.4	88.4	31.0	87.8
1916.....	70.3	51.8	87.2	36.5	86.1
1915.....	70.9	50.7	89.8	29.0	90.4
1914.....	69.8	50.7	87.9	28.0	88.3
1913.....	70.5	51.4	87.5	32.4	93.6
1912.....	70.3	47.8	91.7	30.7	86.3
1911.....	68.0	49.6	86.5	28.9	81.3

Cancer death rates in this present experience, covering six calendar years, and relating in all to fifty million years of life exposed to risk, show no decisive upward or downward tendency for all age classes combined. This is true for each color and sex group, but more decisively for the group of insured white females for whom the highest rates are recorded. The rates, by color and by sex, for the year 1911 are, to be sure, slightly lower than the figures for the entire six year period; this condition may be accidental and without significance. Considering all ages combined, therefore, there is no evidence presented in these figures from which an increasing mortality may be predicated with any certainty.

It would be more significant perhaps in this discussion to consider the trend of the cancer death rate during the six year period in a definite age period, especially one in which the cancer death rate is usually high. For this purpose we have chosen the age period 55 to 64 years. Table 8 shows for each one of the color and sex groups, the death rates from cancer (all forms) during the six year period.

This table shows very much the same trend in the age period 55 to 64 years as we found for all ages combined. The year 1911 was again a year of comparatively low cancer mortality. As the figures are compared for the individual years we find some variation with no clearly defined tendency toward increase or decrease. Our data, therefore, appear to confirm the idea

TABLE 8

Mortality from cancer (all forms) ages 55 to 64 years, classified by color and by sex. Death rates per 100,000 persons exposed. Single years in period 1911 to 1916. Experience of Metropolitan Life Insurance Company, Industrial Department.

Year.	Persons.	White.		Colored.	
		Males.	Females.	Males.	Females.
1916.....	386.4	358.0	427.4	218.3	339.9
1915.....	380.8	336.0	427.8	175.7	394.3
1914.....	390.9	385.0	423.3	167.7	351.7
1913.....	384.1	370.3	414.6	195.2	368.3
1912.....	381.9	334.1	443.2	176.4	325.4
1911.....	368.7	353.3	400.2	158.0	373.7

that the cancer death rate is not increasing, though if such a conclusion were drawn it could be but preliminary. There is need of reserve and caution in predicating any decisive opinion with regard to the real trend of cancer mortality during recent years, for a longer period of time will be required to collect authentic figures upon which a definite judgment can be based. Considerable analysis of cancer data according to age, sex, color, and organ or part affected will, in fact, be necessary before any final conclusions can be drawn as to the amount of increase, if any, in recent years. Another view of our data with respect to this question of cancer mortality increase is presented in the following table:

TABLE 9

Mortality from cancer (all forms). Percentage, death rate per 100,000 persons exposed in 1915-1916 of death rate in 1911-1912. Classified by color, sex and by significant age periods. Experience of Metropolitan Life Insurance Company, Industrial Department.

Age Period.	Persons.	White.		Colored.	
		Males.	Females.	Males.	Females.
Ages 25 and over.....	101.0	105.2	98.3	105.1	100.7
25 to 34.....	98.1	121.0	94.1	95.5	95.0
35 to 44.....	100.1	104.3	96.9	142.6	98.2
45 to 54.....	99.1	109.7	97.0	67.7	93.1
55 to 64.....	102.2	101.1	101.2	118.0	105.2
65 to 74.....	107.0	115.9	101.9	117.2	107.0
75 and over.....	101.0	104.1	104.4	110.7	48.6

We have eliminated in this table any superfluous references to ages under 25 years and in order to get at the heart of the matter have presented only the percentage which the cancer death rate in two years combined, 1915-1916, was of the death rate in two prior years, 1911-1912, at each age period, for each color and sex class.

Considering all persons in this mortality experience at ages 25 and over, there was an increase of only 1.0 per cent in the cancer death rate between the two periods compared. This figure is a composite of a variously weighted increase of 5.2 per cent for white males, a decrease of 1.7 per cent for white females, an increase of 5.1 per cent for colored males and a practically stationary rate for colored females. Considered according to age period, this increase of 1.0 per cent in the cancer death rate of all persons in this experience, aged 25 years and over, was a composite of a decrease of 1.9 per cent between 25 and 34 years, contributed very largely out of the experience of white and colored females, a practically stationary death rate between 35 and 44 years, which is, in itself, a composite of an increase for white and colored males and a decrease for white and colored females, and a slight decrease between 45 and 54 years. At this latter age period, we observe an increase of nearly ten per cent in the white male rate, a decrease of 3.0 per cent for white females, of 6.9 for colored females, and of 32.3 per cent for colored males. The major influence, however, in slightly lowering the cancer death rate of all persons between 45 and 54 years was, of course, the experience of the group of white females. Between 55 and 64 years, all classes in the mortality experience show an increase in the rate, highest for colored males and least for white males. The age period 65 to 74 years shows an increase of 7.0 per cent which is contributed very largely by the experience on male lives of both color groups. It should be remarked that the cancer experience of colored persons exerts but slight influence upon the ratio of increase of cancer mortality in the entire experience. In fact, for some of the age periods, the data on the increase of cancer mortality among colored persons are aberrant.

It will be seen from the foregoing table that considerable analysis of cancer facts according to age, sex, color, and by organ or part affected is necessary before any final conclusions are drawn as to the amount of real increase in cancer mortality, if any, in recent years. A discussion, in some detail, of the cancer mortality experience according to the organs or parts follows.

COLOR, SEX, AND AGE STATISTICS OF CANCER ACCORDING TO
ORGANS OR PARTS

Cancer² of the stomach and liver

The deaths classified under this heading constituted, as was shown above, the most important subordinate group of specific types of cancer. Cancers of the stomach and liver were recorded in 37.6 per cent of all cancers in this entire experience. It should be remembered that this heading also includes cancers and other malignant tumors of the pharynx, the esophagus, and the gall-bladder.³ The combined total of malignant growths of the pharynx and esophagus, however, numbers less than 5 per cent of the total in general practice, and does not, therefore, seriously affect the data for this title heading. In gall-bladder cancers the liver is frequently involved.

During the period of this present investigation, there were recorded 14,153 deaths from malignant growths of the stomach and liver. The death rate was 26.3 per 100,000 persons exposed. As shown in the following table, the facts vary considerably according to color, sex, and age period.

There is a higher death rate for this cause, without important exception, for white lives than for colored lives. Below 35 years, mortality from cancer of the stomach and liver is not numerically important. The death rate increases from a figure of 18.6 in the age period 35 to 44 years to the maximum in the highest age period in this series, 75 years and over. From 45 years of

² Cancer and other malignant tumors.

³ The International list heading "Cancer of the Stomach, Liver," is somewhat misleading, inasmuch as cancers of other organs of the digestive system are classified under it.

age and onward, however, the death rate of white males for this disease is appreciably higher than the rate for white females, with the exception of the very highest age period, 75 years and over. Among colored persons the death rate from cancer of the stomach and liver is higher for males than for females between 55 and 74 years only. At the ages under 55 years, colored females show higher death rates from this cause than do colored males.

TABLE 10

Mortality from cancer of the stomach and liver, classified by color, by sex, and by age period. Death rates per 100,000 persons exposed, 1911 to 1916. Experience of Metropolitan Life Insurance Company, Industrial Department.

Age Period.	Persons.	White.		Colored.	
		Males.	Females.	Males.	Females.
All ages—one and over...	26.3	24.8	29.8	16.2	18.5
1 to 24.....	.3	.3	.3	.2	.6
25 to 34.....	3.0	2.9	2.7	3.3	5.0
35 to 44.....	18.6	18.1	20.1	13.8	16.5
45 to 54.....	67.2	77.0	67.7	44.0	44.8
55 to 64.....	168.5	193.3	168.4	108.0	99.5
65 to 74.....	276.0	303.6	288.7	125.3	122.1
75 and over.....	334.7	339.1	353.3	164.6	217.8

COMPARISON OF DATA FOR CANCER OF THE STOMACH AND LIVER
AMONG INSURED WAGE EARNERS AND AMONG THE POPULATION
OF THE EXPANDING REGISTRATION AREA OF THE UNITED STATES

For both males and females at the ages where death rates from this cause are at all significant, a higher death rate is recorded among the group of insured wage earners than is observed in the Registration Area of the United States. Beginning with the age period 35 to 44 years, there is an excess of over 19 per cent, between 45 and 54 years, an excess of 32 per cent, and between 55 and 64 years, an excess of 29 per cent over the rates for males prevailing in the expanding Registration Area of the United States. The excess in the death rate of cancer of the stomach among insured females is not so great as in the case of insured males. In the following table, a comparison of the foregoing facts is given:

TABLE 11

Mortality from cancer of the stomach and liver. Death rates per 100,000 persons exposed. Insured white lives in experience of Metropolitan Life Insurance Company, Industrial Department (1911 to 1916) and General Population Experience of expanding registration area of the United States (1910 to 1915). Classified by sex and by age period.

Age Period.	Males.			Females.		
	M. L. I. Co. (White).	U. S. Reg. Area.	Per Cent. M. L. I. Co. of Reg. Area.	M. L. I. Co. (White).	U. S. Reg. Area.	Per Cent. M. L. I. Co. of Reg. Area.
All ages—one and over	24.8	30.5	81.3	29.8	32.1	92.8
Under 25.....	.3	.4	75.0	.3	.3	100.0
25 and over.....	65.8	56.4	116.7	63.0	61.4	102.6
25 to 34.....	2.9	3.0	96.7	2.7	3.9	69.2
35 to 44.....	18.1	15.2	119.1	20.1	18.2	110.4
45 to 54.....	77.0	58.4	131.8	67.7	62.1	109.0
55 to 64.....	193.3	150.2	128.7	168.4	147.5	114.2
65 to 74.....	303.6	254.3	119.4	288.7	253.6	113.8
75 and over.....	339.1	294.1	115.3	353.3	313.7	112.6

TREND OF THE DEATH RATE FROM CANCER OF THE STOMACH AND LIVER

Not much stress can be put on the figures showing the trend of the death rate in the period between 1911 and 1916. In the first place, the figures vary considerably from year to year, sometimes increasing, sometimes decreasing; also, cancer of the stomach and liver affects organs which are practically inaccessible for purposes of precise diagnosis. There must, therefore, be considerable uncertainty in the reliability of the diagnosis. It will be necessary, therefore, to wait for an extension in the period of observation before any definite tendency of the death rate from this form of cancer can be predicated. In the meanwhile as diagnostic facilities become more generally available and as the practice of making autopsies becomes more widespread it may be expected that the recorded death rates for cancer of the stomach and liver will show slight increases.

Cancer⁴ of the female genital organs

Cancer of the female genital organs accounted for 28.6 per cent of all cancer deaths among white females. The very largest proportion of these cancers affected the uterus, with the ovaries and Fallopian tubes next in numerical importance.

In all, 7882 deaths from cancer of the female genital organs were recorded in the six year period of this study. The rate has significance only when the details are related to the number of females exposed. The 6499 cancers registered among white females corresponded to a rate of 25.3 per 100,000 such females

TABLE 12

Mortality from cancer of the female genital organs, classified by color and by age period. Death rates per 100,000 persons exposed, 1911 to 1916. Experience of Metropolitan Life Insurance Company, Industrial Department.

Age Period.	White Females.	Colored Females.
All ages—one and over.	25.3	37.9
1 to 19.	.2	.2
20 to 24.	1.0	1.3
25 to 34.	7.9	17.7
35 to 44.	43.4	62.9
45 to 54.	85.7	109.7
55 to 64.	109.4	131.6
65 to 74.	110.3	131.5
75 and over.	93.8	145.2

exposed and the 1383 deaths among colored females to a rate of 37.9 per 100,000 exposed. Under the age of 25 years, there was no significant mortality from this cause. Beginning with the age period 25 to 34 years, however, there was a quite considerable rate of mortality, 7.9 per 100,000 for white females and 17.7 for colored females. This excess in the mortality rate from cancer of the female genital organs among colored females is decidedly marked at all of the age periods in this series. The maximum rates of mortality from this cause appear at the older ages. The preceding table gives the facts according to age classes among white and colored females.

⁴ Cancer and other malignant tumors.

COMPARISON OF DEATH RATES FROM CANCER OF THE FEMALE
GENITAL ORGANS IN INSURANCE EXPERIENCE ON WHITE LIVES
AND IN EXPERIENCE OF THE POPULATION OF THE EXPANDING
REGISTRATION AREA

Again, it is found that white females in wage earners' families show a decided excess in the mortality rate from cancer of the female genital organs over the rates recorded for females in the general population of the expanding Registration Area. The following table gives a comparative view of these death rates:

TABLE 13

Mortality from cancer of the female genital organs. Death rates per 100,000 persons exposed. Insured white females in experience of Metropolitan Life Insurance Company, Industrial Department (1911 to 1916) and females in general population experience of expanding registration area of the United States (1910 to 1915). Classified by age period.

Age Period.	Females.		
	M. L. I. Co. (White).	U. S. Reg. Area.	Per Cent. M. L. I. Co. of Regis- tration Area.
All ages—one and over	25.3	25.0	101.2
Under 25	.4	.5	80.0
25 and over	53.2	47.7	111.5
25 to 34	7.9	8.4	94.0
35 to 44	43.4	34.7	125.1
45 to 54	85.7	75.6	113.4
55 to 64	109.4	99.9	109.5
65 to 74	110.3	103.2	106.9
75 and over	93.8	98.3	95.4

The greatest excess in mortality from cancer of the female genital organs among white females in the families of insured wage earners was recorded between 35 and 44 years. The percentage of excess in mortality from this cause among white females decreases with advancing age, and at the very late ages in life the rates for both the insurance and population experience tend to approximate each other.

TREND OF THE DEATH RATE FROM CANCER OF THE FEMALE GENITAL ORGANS

The figures available show neither a favorable nor unfavorable tendency of the death rate from cancer of the female genital organs. There are slight variations from year to year.

Cancer⁵ of the breast

In view of this very small number of male deaths from cancer of the breast, we shall concentrate our attention entirely upon

TABLE 14

Mortality from cancer of the breast. Number and percentage of total deaths from cancer of the breast in each color and sex class. Experience of Metropolitan Life Insurance Company, Industrial Department, 1911 to 1916

Color and Sex.	Number of Deaths.	Per Cent.
All classes	3,579	100.0
White males	31	.9
White females	3,004	83.9
Colored males	9	.3
Colored females	535	14.9

the statistics for white and colored females. The following table gives the death rates for white females and colored females at the several age periods.

TABLE 15

Mortality from cancer of the breast, females classified by color and by age period. Death rates per 100,000 persons exposed, 1911 to 1916. Experience of Metropolitan Life Insurance Company, Industrial Department

Age Period.	White Females.	Colored Females.
All ages—one and over	11.7	14.7
1 to 24	†	.1
25 to 34	2.5	3.3
35 to 44	17.4	18.9
45 to 54	36.9	41.9
55 to 64	47.6	59.9
65 to 74	76.0	96.3
75 and over	108.8	118.0

† Less than 0.05 per 100,000.

⁵ Cancer and other malignant tumors.

There is a constantly rising death rate with age from this cause for both white and colored females. There is also a decided excess in the death rate among colored over white females although this excess among colored females is not so marked as it was for cancer of the female genital organs.

Throughout the six years under examination, there was, with the exception of the year 1915 for colored women, a practically stationary death rate for cancer of the breast. In the following table, we quote our experience for each of the years from 1911 to 1916:

TABLE 16

Mortality from cancer of the breast, females classified by color. Death rates per 100,000 persons exposed. Single years in period 1911 to 1916. Experience of Metropolitan Life Insurance Company, Industrial Department

Years.	White Females.	Colored Females.
1911 to 1916.....	11.7	14.7
1916.....	11.8	14.9
1915.....	12.5	17.4
1914.....	11.4	12.3
1913.....	11.9	14.3
1912.....	12.1	15.6
1911.....	10.2	13.5

Cancer of the breast seems not to have as heavy a mortality among white insured females as it does among females in the population of the expanding Registration Area of the United States. Between 35 and 44 years, there is practically the same death rate from this cause in both experiences. But between 55 and 64 years the group of insured females in wage earners' families shows much lower death rates from this cause than were found for females in the Registration Area of the United States. It should be recalled at this point that the data for the expanding Registration Area comprise a small proportion of colored women. This fact, considering the higher death rate from cancer of the breast among negro women than among white women, may account in some measure for the higher mortality from this cause in the population experience over the exclusively white insurance experience. The following table affords a comparative view of the statistics for cancer of the breast among insured

white females and among females in the general population of the expanding Registration Area:

TABLE 17

Mortality from cancer of the breast. Death rates per 100,000 persons exposed. Classified by sex and by age period. Insured white females in experience of Metropolitan Life Insurance Company, Industrial Department, 1911 to 1916, and females in general population experience of expanding registration area of the United States, 1910 to 1915

Age Period.	M. L. I. Co. (White).	U. S. Reg. Area.	Per Cent. M. L. I. Co. of Registration Area.
All ages—one and over	11.7	15.5	75.5
Under 25	†	.1	—
25 and over	24.7	29.7	83.2
25 to 34	2.5	2.7	92.6
35 to 44	17.4	17.5	99.4
45 to 54	36.9	41.3	89.3
55 to 64	47.6	61.0	78.0
65 to 74	76.0	81.0	93.8
75 and over	108.8	130.6	83.3

†Less than 0.05 per 100,000.

Cancer^e of the peritoneum, intestines, and rectum

Cancer of the intestines constituted the very largest number of the 4482 deaths under this head. The death rates by age, sex, and color for this cause are shown below:

TABLE 18

Mortality from cancer of the peritoneum, intestines, and rectum, classified by color, by sex, and by age period. Death rates per 100,000 persons exposed, 1911 to 1916. Experience of Metropolitan Life Insurance Company, Industrial Department

Age Period.	Persons.	White.		Colored.	
		Males.	Females.	Males.	Females.
All ages—one and over . . .	8.3	6.6	10.5	3.4	7.2
1 to 24	.4	.4	.3	.2	.3
25 to 34	2.2	1.6	2.2	1.8	3.9
35 to 44	7.8	6.0	8.8	4.0	11.7
45 to 54	22.1	19.7	25.8	7.5	19.9
55 to 64	43.9	43.6	50.5	13.2	21.1
65 to 74	83.1	80.8	95.0	28.4	31.7
75 and over	109.5	86.5	130.6	96.0	36.3

^e Cancer and other malignant tumors.

Mortality from this form of cancer also increases with advancing age. White males show lower death rates at all significant ages than do white females. The colored male death rate from this cause is also decidedly lower than the rate for colored females. It would be well to recall at this present time that in this investigation tuberculous disease of the abdominal organs also shows a higher death rate among females of both white and colored races, especially at the ages of the childbearing period, 15 to 45 years. This fact suggests the possible influence of puerperal traumata as contributing causes in exciting both

TABLE 19

Mortality from cancer of the peritoneum, intestines, and rectum. Death rates per 100,000 persons exposed. Classified by sex and by age period. Insured white lives in experience of Metropolitan Life Insurance Company, Industrial Department, 1911 to 1916, and general population experience of expanding registration area of the United States, 1910 to 1915

Age Period.	Males.			Females.		
	M. L. I. Co. (White).	U. S. Reg. Area.	Per Cent. M. L. I. Co. of Reg. Area.	M. L. I. Co. (White).	U. S. Reg. Area.	Per Cent. M. L. I. Co. of Reg. Area.
All ages—one and over	6.6	8.6	76.7	10.5	10.4	101.0
Under 25.....	.4	.5	80.0	.3	.3	100.0
25 and over.....	16.9	15.5	109.0	21.9	23.5	93.2
25 to 34.....	1.6	2.1	76.2	2.2	2.6	84.6
35 to 44.....	6.0	5.5	109.1	8.8	9.1	96.7
45 to 54.....	19.7	15.2	129.6	25.8	24.6	104.9
55 to 64.....	43.6	37.6	116.0	50.5	51.8	97.5
65 to 74.....	80.8	67.6	119.5	95.0	89.5	106.1
75 and over.....	86.5	80.9	106.9	130.6	117.6	111.1

tuberculous and cancerous processes in the main adult ages among females.

At the ages in which the death rate from cancer of the peritoneum, intestines, and rectum is considerable, insured white males show higher rates than do males in the general population. Between 45 and 75 years of age, the excesses in the rates range from 16 to 30 per cent. Insured white females, on the other hand, show no very marked tendency to depart from the general population experience. At some age periods, the death rate among insured white females is slightly more favorable and at

other age periods slightly less favorable than among females in the general population. The above table gives a comparative view of the data for males and females of the insurance and population experience, considered according to age period.

The data indicate a practically stationary death rate from this cause between 1911 and 1916.

Other forms of cancer⁷

Discussion in great detail of cancers of organs and parts of the body, in addition to those covered by the preceding text, is not justified for many reasons. There are, however, many interesting and important age, sex, and color relations disclosed in the study of "other forms of cancer" which should not be passed without brief comment. These we shall discuss briefly in relation to cancers of the buccal cavity, of the skin, and of all "other organs or of organs not specified" in the order named.

Cancer⁸ of the buccal cavity

Cancer of the buccal cavity, including cancer of the maxillae, caused 1353 deaths during the six year period 1911 to 1916. This corresponds to a death rate of 2.5 per 100,000 exposed. Of the 1353 deaths, 1229 were those of white policyholders and 124 of colored persons. The death rate per 100,000 exposed was 2.6 for white lives as compared with 1.9 for colored lives. Cancer of the buccal cavity shows a very strong sex incidence. Of the 1353 deaths, 1064 were those of males and only 289 those of females. This excess among males applies to both white and colored lives; especially to the former, among whom male mortality (4.6) was more than five times that of females (0.9). White males show a death rate almost twice that for colored males (4.6 as compared with 2.5) and in the female experience also the rate for colored women (1.3) exceeds that for insured white women (0.9). This comparison for colored policyholders, how-

⁷ Cancer and other malignant tumors.

⁸ Cancer and other malignant tumors.

ever, is hardly valid on account of the small number of deaths involved.

The great majority of the deaths classified as due to cancer of the buccal cavity were reported under the terms "cancer of the jaw," "cancer of the tongue," "cancer of the lip," and "cancer of the mouth" without more definite designation.

There is no pronounced upward or downward trend shown for cancer of the buccal cavity in the industrial experience of this Company during the six year period covered by this report. The death rate was 2.3 per 100,000 exposed in the first year of the sexennium as compared with 2.4 for the last year. The rates in the general population are slightly higher than those for the insured. This is accounted for largely by differences in the age distribution of the two populations. As a general proposition only 5 per cent of the deaths from this disease occur among persons under 50 years of age. The great bulk of the deaths are those of men between the ages of 50 and 79.

Cancer^s of the skin

Skin cancers were reported as causes of death in 938 cases in the mortality experience of insured wage earners covering the six year period 1911 to 1916, corresponding to a death rate of 1.7 per 100,000 exposed. Little fluctuation is shown in the rate when the years of the period are compared with one another. Cancer of the skin is, almost altogether, confined to white persons. In the Metropolitan experience 887 of the 938 deaths were those of white persons, the total white death rate being 1.9 per 100,000 exposed as compared with 0.8 for the colored insured. In the matter of sex incidence a considerable excess in the white experience is shown for males over females; the rate for the former being 2.2 as compared with 1.6 for the latter. The rates for colored policyholders are not so significant when compared by sex on account of the small number of deaths involved.

As with cancers of the buccal cavity, only a comparatively small percentum of the deaths from skin cancers are those of

persons under 50 years of age. The mortality is bulked between the ages of 60 and 79 years. When we compare buccal and skin cancers by age groups we find that there is a much higher mortality, relatively, from the skin cancers in extreme old age than from cancers of the buccal cavity.

A lower death rate at all ages for this form of cancer is found among the insured than among the general population. The same explanation for this obtains as for cancer of the buccal cavity, namely, the more favorable age distribution of policyholders. Like that of the insured group, the general population experience for this cause of death shows little change in the rate during the sexennium to which this report relates. The great majority of the deaths charged to this title heading were reported in one of the following ways: cancer of the face, cancer of the nose, cancer of the skin, rodent ulcer, and epithelioma (location not indicated).

CANCER AND OTHER MALIGNANT TUMORS OF OTHER ORGANS OR OF ORGANS NOT SPECIFIED

The above is a *residual* heading under which are classified all deaths from cancers that cannot be definitely assigned to one of the preceding titles; it also includes cancers in which no statement is given of the location or original seat of the disease. The more definite titles included are the cancers of the bladder, of the prostate, of the pancreas, of the kidneys and suprarenals, of the lung and pleura, of the bones (jaw excepted), of the larynx, of the brain, of the testes, of the parotid gland, and of the spleen.

This title constitutes an important cause of death numerically in the present experience. No fewer than 5,279 deaths were charged to it and the corresponding rate was 9.8 per 100,000 exposed. With the exception of cancer of the stomach and liver, and cancer of the female genital organs, more deaths are charged to this residual title than to any of the other separate headings relating to malignant growths.

There was only a very small fluctuation in the death rate during the several years which constitute the period to which

this report relates. This same phenomenon is in evidence for the expanding Registration Area of the United States. The death rate for this cause, however, is considerably higher in the latter experience for these forms of cancer than among the insured group.

The total death rate for the insured white experience was 10.1 per 100,000 exposed as compared with 7.6 for the colored experience. In each, the mortality among males was higher than among females, although this is much more pronounced among the white policyholders than among the colored. This excess for males is accounted for, in part, by the fact that all deaths from cancers of the male genitals are classified under this heading.

REFERENCES

- (1) Mortality from Cancer, etc., Washington, 1914. Published by Department of Commerce, Bureau of the Census, Sam. L. Rogers, Director.
- (2) KNIGHT, AUGUSTUS S. AND DUBLIN, LOUIS I.: The Relation of Cancer to Economic Condition. Read before the Association of Life Insurance Medical Directors of America, Worcester, Mass., October 17, 1917. Reprinted by Metropolitan Life Insurance Company, New York, 1917.
- (3) HOFFMAN, FREDERICK L.: The Mortality from Cancer Throughout the World, Newark, New Jersey, 1915, p. 218; The Mortality from Cancer in the Western Hemisphere, Jour. Cancer Research, 1916, i, 21.
- (4) WILLCOX, WALTER F.: On the Alleged Increase of Cancer. Quarterly Publications of the American Statistical Association, September, 1917, p. 756; Jour. Cancer Research, 1917, ii, 267.

THE LIPOIDS IN TUMORS OF THE DENTAL REGION

KAETHE W. DEWEY

From the Research Laboratory of the College of Dentistry, University of Illinois

Received for publication, November 4, 1918

In view of the enormous amount of investigative work which has been done on the lipoids, and in particular on cholesterol, the contributions to the study of their occurrence in tumors are surprisingly scanty. With the hitherto customary practice of considering most pathological conditions of the teeth and the peridental tissues as somewhat outside the pale of general pathology, tumors of the dental region have received little, if any, attention in the study of this interesting question.

The tumors richest in cholesterol next to cholesteatomas are the hypernephromas; also adenomas of the kidney may contain large amounts of it; cholesterol esters are the chief substance infiltrating xanthoma cells and pseudoxanthoma cells. The pigments to which these tumors owe in part their characteristic color, also the pigments found in chloromas, are considered by some authorities to belong to the lipochromes, that is, to be of a lipid nature.

As elsewhere in pathological and physiological infiltration, cholesterol occurs in tumors as esters and more or less loose combinations with fatty acids. White (1), in examining carcinomas and sarcomas for their content in crystalline substances, found five types of crystals, four of which were cholesterol, either pure or combined, and which could be differentiated by characteristics of the crystalline form and variations in the melting point. An interesting observation to which White has called attention is that gallstones, which consist largely of cholesterol, are two and a half times as common in patients suffering from carcinoma as in patients of the same age suffering from other diseases, and this frequency is independent of the site of the primary

growth. Ciaccio (2) states that cholesterol, or "lecithin" as he calls it, is constantly found in tumor cells and that it is directly related to the malignancy of the tumor, so that it is especially abundant in carcinomas and sarcomas. Podwysotszky (3) reported the finding of cholesterol crystals in an ulcerated cancrroid of the maxilla and suggested that they may be a stage of metamorphic alteration, namely hyaline degeneration passing over into the crystalline state.

The lipid substances discussed in the literature are, as a rule, simply and briefly spoken of as "fat." Although it is now generally admitted that by far the largest part of this so-called "fat" consists of cholesterol in the form of esters or of more or less loose combinations with fatty acids and other lipoids, the infiltration of tissues with these lipoids is still almost universally called "fat" infiltration. For greater precision in terminology, Aschoff (4) proposed the names of "cholesterolester" infiltration and "glycerinester" infiltration as the main types and, in fact, the only ones which come under consideration. Kaiserling (5) adopted a classification of liposis, lipoidosis, and lipo-lipoidosis, according to the substance that is in preponderance. However, this does not clear up the confusion, by any means. Very frequently, cholesterolesters and glycerinesters occur simultaneously in the same tissue and often in the same cell. There is not yet even any unanimity in the use of the terms "lipoid" and "fat." These are applied very loosely by some authorities, while others make sharp distinctions and refer to neutral fat as genuine or ordinary fat. Then there are some who insist on dividing the fatlike substances not into two, but into three classes. That the physicochemical properties, however, are sufficiently marked and that the group distinctions may be made from their reactions to staining methods is best illustrated in tables which Aschoff (4) and Kawamura (6) have published.

Until quite recently the chief stains for lipoids were osmic acid and Sudan III. The latter is an excellent stain and the most satisfactory means to demonstrate all fats or all lipoids, but a differentiation beyond that of the red staining neutral fat

from all the lipoids staining yellowish red cannot be made. As to osmic acid, it is reduced only by oleic acid, olein, and lecithin. Now, however, we possess several new staining methods by which certain lipoids in tissues may be fixed and others eliminated. By employing all these staining methods, and by making use of the polarizing microscope, we are enabled to establish the nature of the different lipoids in tissues with a fair degree of accuracy and in a much larger measure than was hitherto possible. This is the conclusion arrived at in studying tumors and cysts of the dental region for their lipoid content.

The specimens were obtained from Dr. Moorehead's clinic for oral surgery, of the University of Illinois, and included endotheliomas, fibromas, giant-cell sarcomas, cysts and ordinary hypertrophy of the gum tissue.

TECHNIC

Concerning the technic I may briefly state that frozen sections were made from the tissues, fixed in 10 per cent formalin, and stained with Sudan III, Nile blue sulphate, and neutral red. Other pieces and sections were prepared by the methods devised by Ciaccio (7), Smith (8), Dietrich (9), and Fischler (10). These are indispensable in any attempt to differentiate lipoids in tissues. By Fischler's method, fatty acids and soaps are demonstrated. The Smith-Dietrich and Ciaccio methods are based on the observation that in tissues treated with potassium chromate only some of the lipoid substances are chromated, namely the more or less loose combinations of cholesterol with fatty acids and other lipoids. Thus fixed, they are insoluble in fat solvents used for paraffin embedding (Ciaccio), or lake hematoxylin as in Weigert's method of staining the myelin sheath (Smith-Dietrich). The glycerineesters or neutral fat, as well as the stable cholesteroesters, are not fixed by chromium salts. The polarizing microscope reveals the physical properties as follows: cholesteroesters and mixtures of cholesterol with fatty acids and certain lipoids occur in the form of doubly refractive droplets. The glycerineesters, and their mixtures with choles-

terol, fatty acids, and soaps are not doubly refractive. The double refraction disappears on heating and reappears or does not reappear with some lipoids. The melting point varies according to the mixture. Aschoff's and Kawamura's tables are invaluable guides in histomorphologic studies of the lipoids.

STUDY OF SPECIMENS

The largest amount of such substances was found in an angio-endothelioma of the submaxillary gland. The tumor, which was the size of a walnut, was surrounded by a thick fibrous capsule. Cut surfaces showed great mottling, as there were large irregular blackish areas, alternating with gray, spongy-looking tissue and with regions of dense white tissue.

In unstained frozen sections there are many large and small blood-spaces and blood effusions, some of the spaces being empty. Everywhere in these areas there are scattered singly, or in small clumps, yellow, yellowish red, brown, or black erythrocytes and leukocytes. The red cells are often quite homogeneous. The leukocytes are coarsely granular and sometimes disintegrated. The pigmented cell collections are near the margin of the spaces. All spaces have well defined walls. Besides the pigmented cells, there is blackish brown pigment about most of the vessels, often forming a thick coat. Under the low power lens, it looks like iron filings; under the high power lens it is found to be a granular material. These pigmented granules pervade almost the entire tumor tissue and form fine lines about the cells. There is no pigment about the capsule.

In sections stained with Sudan III, there is an amazing amount of orange red material. The tumor cells look as if embedded in a solid trabecular frame-work of lipoid substances. Also the individual cells in large portions of the tumor are encircled by a finely granular yellow material. All the large and small spaces are surrounded by thick coats of the same material in all shades from light yellow to dark orange red. Sudan III corresponds to the fine black material resembling iron filings in unstained sections, but is far in excess of this pigment. A small portion remains

black. The broad connective tissue capsule contains large amounts of a reddish-stained material. The plasma in the blood-spaces is orange red and contains distinct droplets. The structure of the orange red trabeculum is not distinguishable. A conspicuous feature is the large, round, morula-like masses of bright orange red globules lying in spaces, some of which are filled with orange red coagulated plasma. The droplets are small and uneven, and entirely unlike neutral fat droplets. These round morula-like masses are apparently greatly enlarged leukocytes completely transformed into lipid material. There is no nucleus visible, and although they are sharply outlined from the homogeneous protoplasm which has sometimes shrunk away from them, no cell membrane is discernible. Sometimes the lipid globules are dispersed from their original boundary. Very large lipid transformed cells are also found in rows about some vessels, and single red granular cells are scattered in regions of blood effusions. In view of this enormous amount of lipoids throughout the tumor, including the fibrous capsule, it is striking that the tumor cells themselves are free from stainable lipoids.

Nile blue stained sections reveal the same enormous amount of lipoids as sections stained with Sudan III. There is no bright red stained material denoting neutral fat. The trabeculum appears in a lavender color with a reddish tinge. The homogeneous lipid erythrocytes, generally enlarged, are a dirty brownish red, and the huge morula-like leukocytes are lavender. The clumps of yellow granular leukocytes or homogeneous erythrocytes seen in unstained sections remain so in Nile blue stained sections. They are sometimes intermingled with reddish granular droplets. The vessels are surrounded by a purplish, finely granular material. The endothelial cells lining the spaces are filled with fine purplish-red staining granules. They are sometimes slender, sometimes greatly enlarged, and occasionally disintegrated.

There are within the outer zone of the capsule solid, round, or ringlet shaped masses in large numbers which are newly formed blood-vessels in early stages of calcification. They are stained intensely blue or almost black. This deep blue coloring,

indicating fatty acids and soaps, is particularly interesting, because the vessel walls within the tumor tissue proper are not blue, but have shades varying from pinkish lavender to purple.

VARIOUS METHODS AND THEIR RESULTS.

Contradictory views have been expressed concerning the value of Nile blue. Some consider it as unreliable and unsatisfactory, others praise it as the most efficient of staining methods for lipoids. My experience with the stain has been that occasionally it is not practicable; namely, when the lipoids are present in extremely fine droplets and dispersed diffusely within a tissue. But wherever lipoids are present in appreciable amounts and are of different types, Nile blue is superior to Sudan III as a differentiating stain. In tissues very rich in lipoids, as is this endothelioma, one may obtain, almost at a glance, a fairly accurate estimate of the types of lipoids from the scale of shades, from red to deep blue. The coloring of some lipoids seems to change within twenty-four hours from the time of staining, at least in some cases. I have observed that particularly fatty acids and soaps may be decidedly reddish when freshly stained, and may next day appear in the characteristic dark blue tint. Also, the cholesterol mixtures are sometimes more reddish at first and gradually assume the purplish hue. After this initial change, the coloration is stable.

In sections prepared by Smith's method, only a part of the lipoids revealed by Sudan III and Nile blue are demonstrated. They are contained within large cells grouped about vessels and sometimes also in collections within vessels. These cells, leukocytes and endothelial cells, are bluish gray, the typical bright blue coloration not being observed in these sections. The yellow and black pigmented elements and structures are not changed. There are areas within the capsule in which streaks of a faintly bluish gray granular material are present. It is an interesting observation that if sections mordanted by Smith's method are stained with Sudan III, much more of the black material with the appearance of filings reappears in red, than is the case in un-

mordanted formalin-fixed sections. According to Kawamura's table, the lipoids in these sections which are positive with Smith's method may be considered as mixtures of cholesterol with fatty acids. They are anisotropic under the polarizing microscope. Double refraction is lost in heating the sections. These same substances are negative with Fischler's and Ciaccio's methods.

In sections prepared by the latter method, the only lipoids demonstrated are found almost exclusively within the broad connective tissue capsule. They appear as numerous homogeneous circular or crescent-shaped red stained masses which represent the degenerated thickened walls of capillaries. The same structures stain black with Smith's method and dark blue with Nile blue, and are positive with Fischler's staining method; hence they may be considered fatty acids and soaps. In the tissue of the tumor proper, there is very little material about vessels or elsewhere that is positive with Ciaccio's method.

Examination with the polarizing microscope shows that the lipoids occurring as doubly refractive crystals are limited to large cells described in the foregoing (fig. 1) which stain grayish blue with Smith's method, and lie in wreaths around the vessels, or in collections within them. These cells are completely filled with brilliant crystals. Some of them show the central cross before heating. Besides the large cells filled with crystals, there are smaller collections and single globules lying outside the cells. All these crystals disappear on slight heating and reappear on cooling. None of the different kinds of pigments appears as doubly refractive crystals. The lipoids occurring in streaks and masses in the capsule and in the trabeculum of the tumor proper have a brilliant silvery luster. According to Kawamura's table, these doubly refractive crystals, disappearing on heating, are probably mixtures of cholesterol with fatty acids; for cholesteroesters, having the same properties, may be ruled out because they are negative by Ciaccio's, Smith's, and Fischler's staining methods.

The findings in this tumor, therefore, illustrate the fact that all the methods combined for demonstrating the lipoids are adequate means to differentiate most of the lipoids that may be

present side by side in the same tissue. Smith's and Fischler's methods are apparently exceedingly reliable in demonstrating a group of lipoids classified as fatty acids and soaps.

A frequent observation in these tumors of the dental region is the presence of large amounts of lipoids in leukocytes. Apparently complete lipoid transformation of the cells takes place

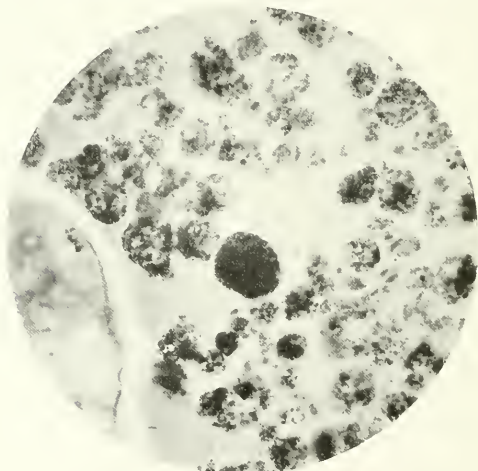


FIG. 1. FROZEN SECTION STAINED WITH NILE BLUE

From an angioendothelioma. Huge leukocytes in a large space containing coagulated serum; they are completely filled with doubly refractive lipid droplets (mixtures of cholesterol with fatty acids). $\times 280$.

in this condition. In the endothelioma described in the foregoing, they are found in large and small collections within blood-spaces, in hemorrhagic areas, in layers encircling blood-vessels, in short, wherever slowing of the blood-stream or stasis has caused a gathering of white cells. The cells filled with lipoids are sometimes enormously increased in size. These and the endothelial cells lining blood-vessels are the only cells in this

tumor which morphologically reveal lipid substances. The tumor cells themselves are free from demonstrable anisotropic droplets under the polarizing microscope. On the other hand, the connective tissue of the fibrous capsule and of the trabecular strands separating the tumor cell nests, when undergoing lipid alterations and transformation, has not doubly refractive properties. In other tumors, like fibromas and papillomas, the doubly refractive droplets filling leukocytes within the small vessels are frequently the chief or even the only lipoids present in the tissue outside the epithelial layer.

A considerable amount of lipoids was also found in a hard or composite odontome. The main substance of this tumor, which was as large as a walnut, was dentin; enamel was present almost exclusively on the outer surface in the form of nodules and scales. Microscopically the dentin was arranged either in irregular solid conglomerations or, more generally, in layers around spaces which represented the pulp chambers. Some of the spaces were filled with cellular tissue resembling that of the pulp, others were occupied, partially or totally, with a more or less homogenous material. Some were empty. A noticeable feature of sections from the tumor in regard to the lipoids therein, was that these substances were almost throughout limited to the regions where there were still some cellular elements, namely to the pulp spaces, and to the dentinal tubules. They occurred in fine granules which stained orange red by Sudan III and purplish by Nile blue. In many regions the tubules of the dentin were filled with such granules. Sometimes a direct connection of these with the lipoids of the pulp spaces was plainly visible. Most of these substances were cholesterolesters or mixtures of cholesterol with fatty acids. There was practically no neutral fat present, and very little of those substances which react to Fischler's staining method for fatty acids and soaps.

In a series of tumors the chief lipoids, as far as the staining methods enable us to determine them, were fatty acids and soaps. These are placed in one group in my study, because their staining reactions are the same. I have made no attempt to ascertain the nature of these compounds. In Kawamura's table,

the reactions for fatty acids are in parenthesis referred to oleic acid, and those for soap, to sodium oleate. Such lipid substances were found within the squamous epithelial covering of papillomas, fibromas, and the mucosa of ordinary hypertrophy of the gum. They were, however, by no means found in all such specimens. The great irregularity in the amount, locality, and distribution of these lipoids is a notable feature; thus it has happened that of twenty sections nineteen were absolutely negative and the only positive one showed a relatively large amount of fatty acid. The site of these lipoids is of interest. In such pathological specimens, the outer layer of the mucous membrane of the squamous cell covering of tumors may present alterations which resemble cornification of the epidermal layer. Such areas take a rosy tint with Sudan III, stain intensely by Fischler's and Smith's methods, and are positive by Ciaccio's. But deeper portions, also, and the innermost layer of the epithelial "pearls," may be distinctly positive with Fischler's, the specific stain for fatty acids and soaps. This finding illustrates well the close relationship of the mucous membrane to the skin, in which lipoids, or "fat," as they have habitually been called, have been found by writers like Unna (11), Sata (12), Karwieka (13) and others. Unna demonstrated a "fat impregnation" in the horny layer of the normal skin. Sata found "fat" chiefly in the rete malpighii. Both reported also the presence of fat in the parietal cells of the vessels of the skin, as well as between the red corpuscles and within lymph-spaces and lymph-vessels. Similar observations have been reported by others. A relationship of eleidin to fatty substances has been suggested. There are others, however, who dispute Unna's assertions. Hagemeister even declares that cornification and fat infiltration exclude one another. He states that this form of necrosis is incompatible with fat-formation.

The largest amount of lipoids demonstrable by Fischler's method was found in papillomas (figs. 2 and 3), and here they were present almost to the exclusion of all other lipoids. They stain deeply blue or almost black, by Fischler's and Smith's methods, and are pinkish with Sudan III. Under the polarizing micro-

scope they appear as most brilliant silver masses. In no instances are doubly refractive droplets observed. Unna called such infiltration in the skin "fat impregnation." Since we know now that the impregnating substance is not neutral fat, "lipoid impregnation" is probably the better name. It is worth mentioning that in the process of being stained by Ciaccio's method,

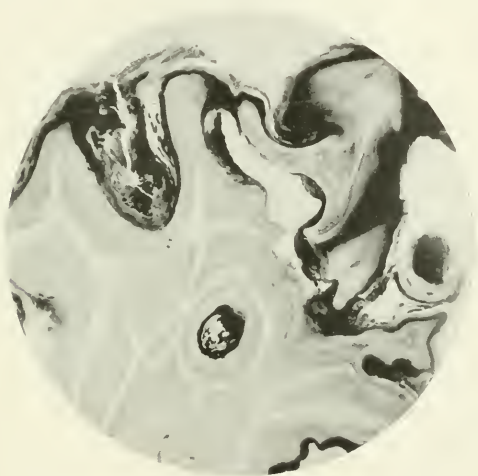


FIG. 2. SECTION PREPARED BY SMITH'S METHOD

From a papilloma, the size of a pea. The black masses, which are dark blue and black in the stained section, represent fatty acids and soaps, all strictly limited to the layer of squamous epithelial cells. $\times 40$.

the regions giving a positive Smith and Fischler reaction take a pink hue with Sudan III, which, however, is crowded out by the subsequent staining with Heidenhain's iron hematoxylin, so that such regions appear finally stained dark blue. Substances giving the same reactions to Fischler's and Smith's methods, to Nile blue, and to Sudan III, and showing the same characteris-

ties under crossed Nicols, were found in tumors without squamous epithelial covering, namely in the walls of vessels within the connective tissue capsule (fig. 4). Hyaline degeneration, so called "fatty degeneration," and calcification were frequently observed vascular alterations. Hyaline degeneration and lipoid "impregnation" were also found on a large scale in tumors about



FIG. 3. SECTION PREPARED BY FISCHLER'S METHOD

From a fibroma. The black masses are dark blue in the stained section; the lipoid substances (fatty acids and soaps) in this region occupy all the layers of the squamous epithelial covering. $\times 40$.

slits and spaces filled with cholesterol crystals. It would seem that these degenerative changes associated with the occurrence of fatty acids and soaps are in some way, directly or indirectly, connected with calcium deposition. But generally accepted views concerning the processes involved in calcification are still lacking. There is not yet any final explanation of just why cer-

tain hyaline degenerated tissues bind calcium. Fatty acids do not normally occur in tissues as such. Pathologically, however, they are observed in necrotic foci, especially of the lung, of the lipid tissue of the pancreas, of abscesses, and of tumors. Calcium soaps, according to Aschoff, are being more frequently

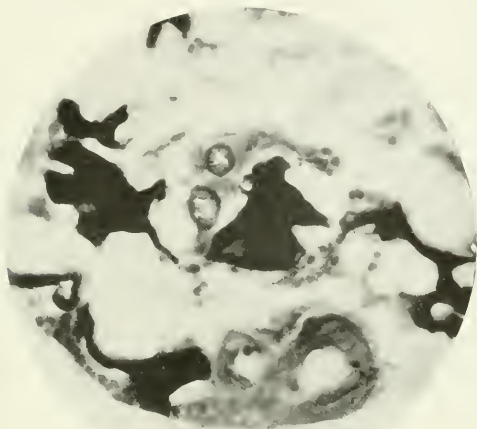


FIG. 4. SECTION PREPARED BY CIACCIO'S METHOD AND STAINED WITH NILE BLUE

From the thick connective tissue capsule of an angioendothelioma, the bundles of which are separated and the spaces occupied by small thick-walled vessels and blood effusions; the black areas are homogeneous masses stained blue in the section and represent the degenerated blood vessels (fatty acids). Four well preserved small vessels are in the same field. $\times 475$.

observed. They occur in arteriosclerosis, in the border zone of infarcts of the kidney, in caseous regions, and, in short, wherever processes lead to calcification. Calcium deposition is not uncommon in tumors. It is observed especially in tumors of the endothelial and epithelial cell type, and here it occurs not only in concentrically placed dead endothelial cells, in cornifying and colloid degenerated cells, but also in the hyaline substance ex-

creted by the cell. Aschoff (14) refers to a close relationship of hyaline and colloid substances to fibrin, which fact seems to explain, he states, why fibrinous hyaline masses, resulting from simple coagulation and coagulation necrosis, degenerated epithelial cells, and hyaline degenerated fibrous intercellular tissue, take up calcium with such readiness.

FATTY ACIDS AS A FACTOR IN CALCIFICATION

I have not entered into an investigation of the nature of soaps and fatty acids in tumors and their relationship to calcification, this being a question beyond the scope of this paper. Although it seems to suggest itself that the same areas which present degenerative changes associated with lipoid deposition may at the same time be the seat of calcareous infiltration, because of the presence of these lipoid substances, the methods which I employed are not the same to prove or disprove any direct relationship of the lipoids to calcification. The opinions concerning this question are divided. Klotz (15) and Aschoff (14) believe that the fatty acids are the "Kalkfänger," i.e., substances which bind calcium from the blood, and that the formation of such soaps is the stage preceding calcification. Subsequently the fatty acids may be replaced by carbonic and phosphoric acids. It is generally admitted that this is the manner in which calcification takes place in fat necrosis of the pancreas and of lipomas. Whether such a process also plays a part in calcification otherwise is not yet determined. Wells (16) denies it. From the results of chemical analyses of normal and pathological material, he came to the conclusion that fatty acids are seemingly not an important factor in the binding of calcium in pathological calcification, and that there is no evidence that calcium soaps form a constant and important stage in the process of calcification. Degeneration and the presence of lipoids, however, are prominent features in areas which are to become calcified. An essential difference between pathological calcification and normal ossification is that in the former the vitality of the tissue is lowered or totally lost and that therefore any cell or tissue may be involved, provided it has degenerated sufficiently, while ossifica-

tion is accomplished only in varieties of connective tissue. In calcification, the significance of the presence of lipoids in degenerated areas is illustrated by Wells, when he refers to differences in healing caseous tubercles and gummas. The former always show much fatty degeneration and almost invariably become calcified when they heal, while gummas are relatively little involved in fatty degeneration and usually do not become calcified.

SOURCE AND SIGNIFICANCE OF CHOLESTEROL

A predominant interest has been attached to the question of the source and the significance of cholesterol infiltrating the cells. Lipoids are a normal constituent of all cells. Physiologically they may be present in large quantities in the liver cells and in the suprarenals; these organs seem to play an important part in the cholesterol regulating metabolism. The adipose tissue is the chief depot in which the lipoids are stored and from which they are conveyed to other tissues in physiological and pathological conditions. In this transport by way of the blood and lymph they have not the physicochemical constitution in which they reside in the cells, but are split into their components and in the form of a solution enter the new cells, there to be reconstructed synthetically. The transmission of lipoids by the blood or lymph may be said to have been conclusively demonstrated by experimental investigations, chiefly those of Rosenfeld (17). Hagemeister (18), on the other hand, claims to have evidence that the source is local. According to him lipoids are liberated from the infiltrated cells in the process of tissue necrosis, and are taken up by the transudate, through which they are imparted to neighboring cells. The transmission is entirely local. It will be admitted that unquestionably such conditions may take place; cells may take up lipid substances directly from their environment; von Recklinghausen (19), Beneke (20), and Arnold (21), have shown this by animal experiments. Also histological studies of emboli, encephalitic foci, give evidence of a direct intake. But there is nothing to prove that this is the only one or is even frequent. Hagemeister's theories as to the significance of cholesterol and the lipoids are not generally

shared. He believes he has demonstrated that lipoids are found only and regularly when there is necrosis; he goes a step further and states that "fat" may be taken up into dead cells, and that on the other hand, increased cellular activity, hyperemia, and proliferation are even instrumental in causing the disappearance of lipoids. These observations have not been confirmed by other investigators. In fact, White states that the cholesterol combinations which he studied by the polarizing microscope occur chiefly in the proliferating areas of tumors. According to him, this location is significant. As cholesterol normally and physiologically plays an important part in the economy and is by no means a waste product, so in tumors it may have an important function to perform. The peculiar location would suggest that it may, in some way or other, be associated with the regulation of proliferation. I may remark here that Hagemeister's failure to observe lipoids in localities where others have found them is most probably due to the staining method he employed. Osmic acid, which he used exclusively, is a stain which by no means uncovers all lipid substances. As is well known, it is reduced only by oleic acid, olein, and so-called lecithin. Some of his deductions must therefore be regarded as disputable, because they are made from incomplete observations. Contrary to his views lipid deposition in cells is considered to be an entirely vital process, although its presence is often a sign of existing degenerative alterations. In tumors such as carcinoma, sarcoma, lymphosarcoma, and adenoma, it is often associated with regressive changes, but is not found in the necrotic areas. Anisotropic droplets do not occur in dead cells. Lipoids may be found in cells without nuclei, but they were there before the death of the cell occurred.

According to Aschoff (4) the storing of lipid substances by cells in the neighborhood of dying tissue, especially in tumors and certain abscesses, is nothing else but a process of resorption of doubly refractive substances which have been set free. There may be marked degenerative processes in cells infiltrated with cholesterol, but the very presence of this substance is a sign that they are still alive, and still active. It also indicates that it is not a mere waste product, but may be a substance involved

in some active function. Rosenfeld even expresses the view that it is of a regenerative character.

The occurrence of these substances is apparently dependent on the blood supply. A characteristic feature of the infiltration in the liver, for example, is the arrangement of the anisotropic droplets along that side of the liver cells which is nearest to the blood-capillaries. Local anemia leads to the so-called fatty degeneration; the cholesterol mixtures, however, are found only in those regions where there are still some tissue fluids circulating, for example, in infarcts within the outer zone. In tubercles, also, they are not in the central region of necrosis, but in the peripheral zone of epithelioid cells. Even in the giant cell the homogeneous protoplasmic portion is free from cholesterol. The lipid is found only about the peripherally placed nuclei. The analogy in the arrangement of the lipid substances in the giant cell and the tubercle is interpreted by Herxheimer (22) to indicate that the central portion free from nuclei in the giant cell, may be considered as undergoing necrosis.

The presence of cholesterol, therefore, far from being a sign of disease, denotes life and activity. Cells very seriously injured, although still alive, seem to be unable to store lipoids.

SUMMARY

1. The selective staining methods of Ciaccio, Smith, and Fischler, used with the general lipid stains, Sudan III and Nile blue, and reinforced by the aid of the polarizing microscope, are fairly adequate means for a histomorphological differentiation of the various lipoids present in tissues.

2. The lipoids in pathological dental tissue are chiefly cholesterol, occurring as the stable ester compound, but much more frequently in more or less loose combinations with fatty acids and other lipoids. The fatty acids and soaps which are found in certain tumors, are located chiefly in the zone of squamous epithelial cells. Otherwise, they occur in the degenerated walls of blood-vessels in tissue with a depleted blood supply.

3. The mixtures of cholesterol with fatty acids occur in the form of droplets and granules within cells, chiefly endothelial

cells and leukocytes. They have anisotropic properties. There are mixtures of cholesterol with glycerinesters and probably other lipoids in degenerated connective tissue which have not the form of droplets and granules and are not doubly refractive.

4. The lipoids in pigments seem to be chiefly mixtures of cholesterol with glycerinesters. Double refraction is not observed in pigments.

5. Fatty acids and soaps are found in areas where hyaline degeneration and calcification also are observed. The question of whether, or how, these substances may be involved in the process of calcium deposition has not been approached in the practical part of this study.

REFERENCES

- (1) WHITE: Jour. Path. and Bacteriol., 1909, xiii, 4.
- (2) CIACCIO: Centralbl. f. allg. Path. u. path. Anat., 1909, xx, 771.
- (3) PODWYSSOTZKY: Grundzüge der allgemeinen Pathologie, 2nd edition, 1898, v. 1 (abstr. in Lubarsch-Ostertag, *Ergebn. d. allg. Path.*, 1898, v. 713).
- (4) ASCHOFF: Zieglers Beitr., 1910, xlvii, 1.
- (5) KAISERLING: Berl. klin. Wehnschr., 1910, xlvii, 2156.
- (6) KAWAMURA: Die Cholesterolverfettung, Jena, 1911.
- (7) BELL: Jour. Med. Research, 1911, xix, 539.
- (8) SMITH: Jour. Path. and Bacteriol., 1908, xii, 1.
SMITH AND MAIR, *ibid.*, p. 134.
- (9) DIETRICH: Verhandl. d. deutsch. path. Gesellsch., 1910, xiv, 263.
- (10) FISCHLER: Centralbl. f. allg. Path. u. path. Anat., 1904, xv, 1913.
- (11) UNNA: Monatschr. f. prakt. Dermat., 1898, xvi, 601.
- (12) SATA: Zieglers Beitr., 1900, xxvii, 555.
- (13) KARWICKA: Virchows Arch., 1906, clxxxiv, 414.
- (14) ASCHOFF: Lubarsch-Ostertag, *Ergebn. d. allg. Path.*, 1902, viii,¹ 561.
- (15) KLOTZ: Jour. Exper. Med., 1905, vii, 633.
- (16) WELLS: Jour. Med. Research, 1906, N.S. ix, 491.
WELLS AND BENSON: *ibid.*, 1907, N.S. xii, 15.
WELLS AND MITCHEL: *ibid.*, 1910, N.S. xvii, 501; Arch. Int. Med., 1911, vii, 721.
- (17) ROSENFELD: Verhandl. d. Cong. f. inn. Med., 1897, xv, 427.
- (18) HAGEMESTER: Virchows Arch., 1903, clxxii, 72.
- (19) VON RECKLINGHAUSEN: Handbuch der allgemeinen Pathologie des Kreislaufes und der Ernährung, 1883, chapter xv.
- (20) BENEKE: Zieglers Beitr., 1897, xxii, 342.
- (21) ARNOLD: Virchows Arch., 1903, clxxi, 197; Centralbl. f. allg. Path. u. path. Anat., 1905, xvi, 481.
- (22) HERXHEIMER: Lubarsch-Ostertag, *Ergebn. d. allg. Path.*, 1902, viii,¹ 681.

THE SIZE OF THE SPLEEN IN IMMUNE MICE¹

WILLIAM H. WOGLOM

*From Columbia University, George Crocker Special Research Fund, F. C. Wood,
Director*

Received for publication, February 11, 1919

HISTORICAL REVIEW

It has been asserted by not a few authors in recent years that the spleen is enlarged in mice bearing, or immune to propagable tumors; and implied, if not actually stated, that the hypertrophy is an expression of the important part taken by this organ in the elaboration of protective substances of all sorts. This involves the subsidiary assumption that immunity to transplantable new growths is due to some sort of antibody, though it is admitted that no evidence of the presence of any antibody similar to those active against the various bacteria has yet been advanced.

Cimoroni (1), Brancati (2), Price-Jones (3), and Mazza (4), have all recorded enlarged spleens in mice or rats bearing transplantable tumors. Thus Brancati wrote that whereas in the normal animal the relation of spleen weight to body weight is as 1 to 169, in those with tumors it is as 1 to 60. Mazza asserted that the spleen is enlarged in rats developing small multiple nodules after intraperitoneal inoculation, but of normal size in those with a single large growth. Price-Jones found that the two heaviest spleens in his series occurred in the two mice with the largest tumors, and that there was about twice as much myeloid activity going on in the spleens of cancer mice as in those of normal ones. In a later paper (5) he described a group

¹ A preliminary report of these investigations was presented at the Tenth Annual Meeting of the American Association for Cancer Research, New York, April 5, 1917. See *Jour. Cancer Research*, 1917, ii, 501.

of rats bearing transplantable sarcoma. The spleen had increased 56 per cent in weight in rats with non-ulcerated tumors, 115 per cent in the animals whose tumors had undergone slight ulceration, and 130 per cent in those with extensive ulceration. As there were only two rats with non-ulcerated growths, he left open the question whether the spleen is enlarged in animals bearing non-ulcerated neoplasms.

Apolant (6), on the contrary, declared definitely that the splenic enlargement in tumor-bearing mice is not due to ulceration, for he found that in animals with old and deeply ulcerated neoplasms (especially sarcomata) the spleen is extraordinarily small. Apolant asserted, furthermore, that this organ is enlarged not only in mice bearing spontaneous or transplanted growths, but also in those that have been unsuccessfully inoculated with tumor, or immunized with normal tissue.

A second reason for crediting the spleen with an active rôle in protecting the body against transplanted cancer has been the observation, frequently recorded by pathologists, that metastases are rarely found in this organ. Still, there is good reason to believe that this is not solely because it offers a soil antagonistic to the growth of the cancer cell, but because the cancer cell often does not find itself amid suitable mechanical conditions. Within the past few years v. Hansemann (7) has emphasized his belief that metastases are not so rare in the spleen as they are ordinarily supposed to be, and Kettle (8) has ascribed what freedom the spleen does enjoy, if any, to the contractions of this organ, which force tumor emboli out of it or prevent the growth of such as have already succeeded in becoming impacted in its capillaries. Finally, the argument is clinched by the observation of Graf (9), Goldmann (10), and Endler (11), that in animals tumor grafts grow after having been inoculated into the spleen. In the experiments of Graf, indeed, 84 per cent of such grafts were positive, an outcome that compares favorably with the result following subcutaneous transplantation. Even Brancati (12), a believer in the immunological importance of the spleen, admits that tumor grafts will grow in this organ, though it is true that he says the proliferation is more restricted than at other sites.

A third reason that has been advanced to uphold the importance of the spleen as a protective factor is the assertion of Apolant that the resistance of an immune animal can be abrogated by splenectomy (13), and that it is difficult to immunize splenectomized animals; the fact that they can be immunized at all, however, suggested to him that the spleen cannot be the only organ involved in the elaboration of resistance. Both these statements have been challenged by Bullock and Rohdenburg (14), who found that removal of the spleen does not interrupt an existing immunity, and that splenectomized mice are made resistant as easily as any others.

A fourth claim has been entered for the spleen, in the assertion that tumors grow more readily in splenectomized animals because of the removal of its protective action (Oser and Pribram (15); Brancati (16)). But Rohdenburg, Bullock, and Johnston (17), Biach and Weltmann (18), Morris (19), and Mottram and Russ (20), have all found that splenectomy neither increases nor decreases the receptivity of normal animals for tumor grafts. Mottram and Russ say, however, that when immune rats are splenectomized, some microscopic growth of the tumor results. With the idea that any increase in susceptibility, however slight, would be best shown after inoculation with a tumor giving a low percentage of takes, Bullock and Rohdenburg (21) transplanted spontaneous mouse tumors into a series of splenectomized mice, but found them no more receptive than normal controls.

Finally, as a fifth argument for the participation of the spleen in the immune reaction, it has been asserted that treatment of tumor-bearing animals with the spleens of mice that have been intraperitoneally or subcutaneously injected with tumor will bring about a recession of their growths (22). The explanation of this statement lies in Braunstein's naive remark, that the results seem to have been better with sarcoma; for it is now well known that transplantable sarcomata regress without any treatment in a large percentage of the animals bearing them. Furthermore, Morris (23), who has but recently investigated this question with great care and on a large series of animals,

was quite unable to discover the slightest effect upon transplantable mouse sarcoma after injecting the animals with the spleen or blood of normal, tumor-bearing, or immune mice.

A closely related assertion is that of Mottram and Russ (24), and Biach and Weltmann (25), that spleen will inhibit the taking of a rat sarcoma where it is mixed with the tumor before inoculation. Both papers contain the statement that other organs will not do this, and that the effect is especially distinct when spleen from tumor-bearing rats is employed, invoking as an explanation the presence in this tissue of some specific substance able to damage the tumor cell. Mottram and Russ, also, seem somewhat inclined to accept a similar explanation; at any rate, they say that the result is not to be ascribed to any general immunizing action exerted by the spleen, for when tumor is inoculated on one side of the animal and spleen on the other, no effect is observed. These latter authors describe, also, a great increase in the total number of lymphocytes and plasma cells in the spleens of most immune rats, a reaction which they compare with that described by Da Fano (26) in the subcutaneous tissues.

The preceding brief review of the question shows that almost no evidence has yet been advanced for the significance of the spleen in resistance that cannot be matched with equally good evidence against its participation; so many are the unknown factors in the equation which the investigator of cancer is called upon to solve.

CAUSES OF ENLARGEMENT

It is not the purpose of the present paper to deny that the spleen has any part in the production of the refractory state, but only to show that there is, so far, no incontrovertible proof that it has; and, particularly, to demonstrate that enlargement of the spleen in mice does not prove that this organ participates in bringing about resistance to the inoculation of a tumor.

Underlying all the reports of an hypertrophied spleen in tumor-bearing or immune mice is the tacit assumption that the organ was of normal size before inoculation. But this supposition is quite unwarranted, for the spleen is frequently enlarged

in mice that have received neither an immunizing injection nor a tumor graft, being sometimes eight times the normal size (27). In the white rat, too, it may often be several times the normal weight (28). Yet only a few investigators appear to have been aware of this, though the wide differences in so-called normal animals were described some years ago by Medigreceanu (29). Because of its variable size, Medigreceanu was unwilling to say whether or not the spleen shares in the enlargement which he discovered to involve other organs (heart, liver, and sometimes the kidney) of tumor-bearing mice. If it does, its increase may be only a part of the general hypertrophy, and may actually help to provide for, and not antagonize the tumor, as Bashford (30) has pointed out.

Continuing Medigreceanu's investigation, Bashford has shown that hypertrophy of the spleen, if any, is greater in animals with progressively growing neoplasms than in those where the growth has been absorbed, so that the enlargement must be independent of the resistance. Finally, Rondoni (31) has insisted that hypertrophy of the spleen in tumor-bearing animals is not to be referred to any protective action exerted by this organ.

To what, then, may it be ascribed? In the mouse, at least, often to mouse typhoid. Many years ago Loeffler (32) found the spleen almost constantly enlarged in this disease, and the writer can testify to the wide distribution of mouse typhoid among white mice, in the vicinity of New York at any rate. A spleen of normal size and healthy pink color is, in consequence, rather a rare finding in adult mice, most of those examined being swollen and of a purplish hue. Furthermore, E. G. Cary of this laboratory, after an investigation of mouse typhoid and possible methods of controlling it, came to the conclusion that the majority of mice have become infected even before they are shipped from the breeding places, that is, before reaching the age of two or three months, and that stock from every dealer, within a hundred miles of New York City at least, will contain infected animals at one time or another. I wish at this point to thank Dr. Cary for his kindness in allowing me thus to refer to his still unpublished work.

The ubiquity of mouse typhoid makes it evident that statements describing enlargement of the spleen in tumor-bearing or immune mice can no longer be accepted as valid unless the size of the spleen before inoculation was known.

NORMAL SIZE OF SPLEEN

The normal volume of the spleen is unknown, as has already been intimated, but it is in the neighborhood of 0.1 cc. The authority for this supposition rests upon measurements made on five mice, three of which had been kept isolated, each in a cage alone, since they were weaned, so that the chances of infection were reduced to a minimum. In these three mice, which were eight, nine, and twenty months old respectively, the measurements were: $1.4 \times 0.35 \times 0.1 = 0.049$ cc., $1.45 \times 0.45 \times 0.15 = 0.098$ cc., and $1.7 \times 0.5 \times 0.15 = 0.127$ cc. These volumes correspond closely enough with those for the remaining two of the five, young adults which, though they had not been isolated, were found to have spleens of healthy appearance as contrasted with the swollen purplish look so often encountered. Here the dimensions were $1.3 \times 0.45 \times 0.1 = 0.058$ cc., and $1.7 \times 0.55 \times 0.17 = 0.159$ cc. The average of the five volumes is 0.098 cc.

METHOD OF MEASUREMENT

The remainder of this paper will describe a series of experiments in which the size of the spleen was known before immunization or tumor inoculation. It was thought at first that a fairly exact idea of the dimensions of the organ might be had by measurement through the shaved and wetted skin; but when figures so gained were compared with the results of direct measurement immediately afterward, the mouse having been killed in the meantime, it was discovered that the error was so high as to demand direct measurement of the spleen by laparotomy; all the figures employed are accordingly the product of this latter method.

Under ether, an incision about 1 cm. in length was made,

through which the spleen was drawn. The length, breadth, and thickness of the organ were then measured in centimeters with a slide caliper to the nearest 0.05 cm., the spleen was replaced in the abdomen, and the wound repaired with one continuous suture. Owing to the curvature of the spleen, the length as measured was somewhat less than the actual length; but the organ was laid as flat as possible on the abdomen, and the error was about the same in each mouse, so that the results are fairly comparable. The method is not accurate in any case, for the breadth and thickness vary somewhat in any one spleen, and all that can be done is to select what appears to be the average dimensions; it is unfortunate that the nature of the experiment made it impossible to employ the weight of the organ, rather than its volume.

SOURCES OF ERROR

In measuring a yielding structure like the spleen, it is obvious that there will be some personal error. To determine its magnitude, a dozen mice were killed and all twelve spleens measured once in their three dimensions. The organs were then measured a second time and the results recorded on another slip of paper; the length of time consumed by the first measurement (twenty minutes) and the number of dimensions recorded (thirty-six) precludes the possibility that any of the results were remembered when the second series of measurements was made. In six of the cases the second measurement was larger, in five smaller, and in one the two results coincided. The greatest positive error (second measurement larger) was 44 per cent, and the greatest negative was 20 per cent. An error of 44 per cent, however, is really smaller than it seems, as attention to the six measurements which produced it will show; thus the results of a first measurement were: $2.0 \times 0.6 \times 0.15 = 0.180$ cm., and of a second: $2.0 \times 0.65 \times 0.2 = 0.260$ cm. A difference of 0.05 cm. in each of two diameters was therefore sufficient to bring it about. Still, if the error happened once in twelve cases it might have occurred about eight times in a hundred, and hence it cannot be disregarded. It will accordingly be best to acknowledge frankly

that the personal error in all the following measurements is, in round numbers, 50 per cent and to exclude from the number of enlarged spleens all such as have not increased more than 50 per cent in volume. As the augmentation is often over 500 per cent the elimination of spleens with less than 50 per cent enlargement is not such a radical excision as it might appear to be at first sight.

In order to be sure that measurements of the spleen made during life at laparotomy are comparable with those made after death, the spleens of two mice were measured under ether anaesthesia. The animals were then killed and the spleens measured again about fifteen minutes after death. No difference was found, and it is a legitimate conclusion, therefore, that the post mortem measurements in this paper, all of which were made within fifteen or twenty minutes after death, may be compared or contrasted with others made during life.

But an essential preliminary to any such procedure is a knowledge of whether the spleen in mice subjected to laparotomy and measurement of this organ, and otherwise untreated, will enlarge during the subsequent few weeks as a result of the operation or of other causes inseparable from the conditions governing the experiment.

Table 1 represents the survivors of thirty-six mice whose spleens were measured on December 13, 1918. These thirty animals were killed, and their spleens were measured again, two weeks later, on December 27, 1918. As in tables 2 and 3, the upper figures for each mouse give the volume in cubic centimeters at the first measurement, while the lower are the volume at autopsy. The percentage is the percentage of enlargement; where it has been omitted, the spleen at autopsy was the same size as at the first measurement, or smaller. In the latter case, the organ is to be regarded merely as not having been enlarged, consideration of its shrinkage lying outside the domain of the present discussion.

Of the thirty mice in the table, ten had spleens that were not enlarged at autopsy. In the remaining twenty, six showed an enlargement exceeding the personal error of 50 per cent. There-

fore, of a group of mice laparotomized and set aside for two weeks, about 20 per cent developed an hypertrophy of the spleen.

In a second experiment (table 2), a longer period was allowed to elapse between the first and second measurements. The spleens of thirty-six mice were measured on April 28, 1916, and

TABLE 1

MOUSE	VOLUME OF SPLEEN	ENLARGE- MENT	MOUSE	VOLUME OF SPLEEN	ENLARGE- MENT	MOUSE	VOLUME OF SPLEEN	ENLARGE- MENT
	<i>cc.</i>	<i>per cent</i>		<i>cc.</i>	<i>per cent</i>		<i>cc.</i>	<i>per cent</i>
1 {	0.108 0.120	11	13 {	0.187 0.222	19	25 {	0.187 0.195	4
2 {	0.101 0.170	68	14 {	0.112 0.148	32	27 {	0.144 0.153	6
3 {	0.127 0.190	50	15 {	0.101 0.142	142	28 {	0.153 0.131	
6 {	0.216 0.116		16 {	0.148 0.076		29 {	0.112 0.260	132
7 {	0.204 0.198		17 {	0.105 0.170	62	30 {	0.120 0.072	
9 {	0.132 0.160	21	18 {	0.094 0.101	7	31 {	0.176 0.116	
10 {	0.058 0.127	119	20 {	0.162 0.127		32 {	0.160 0.160	
11 {	0.090 0.131	45	21 {	0.198 0.240	21	33 {	0.200 0.210	5
12 {	0.127 0.102		22 {	0.094 0.198	111	34 {	0.132 0.170	29
			23 {	0.170 0.214	26	36 {	0.432 0.135	
			24 {	0.198 0.214	8			

Mice laparotomized and set aside for two weeks. About 20 per cent have developed an hypertrophy of the spleen.

the animals were killed on June 14, 1916—forty-seven days later. In seven of the thirty survivors, the spleen had not enlarged. Of the remaining twenty-three, fourteen showed an enlargement of more than 50 per cent. Therefore, of a group of mice laparotomized and set aside for about seven weeks, approximately 50 per cent developed an hypertrophy of the spleen. Not only

TABLE 2

MOUSE	VOLUME OF SPLEEN	ENLARGE- MENT	MOUSE	VOLUME OF SPLEEN	ENLARGE- MENT	MOUSE	VOLUME OF SPLEEN	ENLARGE- MENT
	cc.	per cent		cc.	per cent		cc.	per cent
1	0.136 0.190	40	14	0.096 0.252	162	27	0.166 0.367	121
2	0.116 0.127	9	15	0.121 0.064		28	0.124 0.132	6
3	0.093 0.092		16	0.090 0.121	34	30	0.228 0.308	35
4	0.108 0.252	133	17	0.049 0.200	308	32	0.073 0.200	174
5	0.054 0.038		18	0.175 0.135		33	0.042 0.153	264
6	0.048 0.087	81	19	0.093 0.190	104	34	0.294 0.308	5
7	0.252 0.118		20	0.090 0.102	13	35	0.040 0.132	230
8	0.127 0.294	131	21	0.038 0.036		36	0.116 0.220	90
10	0.200 0.264	32	22	0.058 0.090	55			
11	0.013 0.120	823	23	0.228 0.231	1			
12	0.060 0.264	340	24	0.140 0.124				

Mice laparotomized and set aside for nearly seven weeks. About 50 per cent have developed an hypertrophy of the spleen.

does the number of mice so affected increase with time, but the enlargement is greater. Thus, the largest spleen in this series had undergone an increase of 823 per cent, as compared with 142 per cent after two weeks. It cannot be objected that in the latter instance the organ had reached dimensions beyond which it is impossible to increase, for its final volume was but 0.142 cc., whereas spleens over 0.300 cc. in volume can be found in both tables.

It is impossible to prove that the hypertrophy in the two preceding experiments was due to cage infection rather than to infection at operation, but the following facts strongly support the view. The mice in tables 1 and 2 are arranged according to their disposition in boxes; column 1 in each table contains survivors of the twelve mice of Box I, column 2, those of Box II, and so on. The individual mice are numbered in the order in which they were operated upon, the missing numbers, of course, representing those that died before the experiment was terminated. The mice in Box I were always laparotomized in the morning, and those of Boxes II and III at one sitting in the afternoon, the instruments being sterilized when Box I was begun, and once again for both Boxes II and III. Immersion in alcohol was relied upon to keep the instruments reasonably clean between individual operations. Now if infection at operation were the cause of the splenic enlargement, it should become increasingly evident in the higher numbers of Boxes I and II, and should be greatest in Box III, since the instruments had been used on twelve mice before this box was begun. Yet in table 1, only one of the ten mice in Box III (last column on right) had a spleen enlarged more than 50 per cent of its size at the first measurement. Again, the three mice that died in Box I were not Nos. 10, 11, and 12, as might have been expected had infection played any considerable part in their subsequent history, but Nos. 4, 5, and 8. Similarly in Boxes II and III, the last mice to be operated were not the ones that died (with the single exception of No. 35 in Box III). A similar discrepancy between the chances of operative infection and the occurrence of death is to be noted in table 2; nor is there any greater tend-

ency here than in table 1 for the mice last operated upon to have large spleens. It is unnecessary to labor the point, for in no way can a positive proof be adduced from the data at hand, but it seems fair to conclude that laparotomy does not result in enlargement of the spleen, when done with reasonable cleanliness.

Infection with the *B. typhi murium*, on the other hand, will cause the spleen to hypertrophy, as table 3 demonstrates. A group of mice with measured spleens were inoculated with an organism recovered at this laboratory. Except for an isolated case (mouse 4), there appears to be little or no increase in the volume of the spleen before the eighth day, where the dose was 0.05 cc. of a two-loop emulsion. At this time, some were enlarged over 50 per cent of their volume and some were not, but by the thirteenth day there was considerable increase in size. Where 0.1 cc. of emulsion was injected, the enlargement was greater and set in earlier.

It is quite permissible, therefore, to refer the splenic enlargement noted in table 1 to cage infection with the bacillus of mouse typhoid, since it has been proved that nearly all mice suffer from this disease, and that its bacillus will cause the spleen to hypertrophy.

Before going further, it will perhaps be advantageous to review the sources of error so far discussed. First, the normal size of the mouse spleen is not accurately known; secondly, there is a personal error in measuring this organ which may amount to about 50 per cent; thirdly, the spleens of mice kept in a cage with other mice will often undergo enlargement.

Variations in the size of the spleen brought about by its rhythmic contractions do not enter as a source of error, for they do not exceed 20 per cent of its volume, at least in the dog and cat. There is no reason to suppose that they are two and a half times as great in the mouse, and they therefore come, in all probability, well within the personal error. But even though they do not, the error is the same throughout the experiment, and, by reason of the large number of measurements, may be disregarded.

Finally, because the relation between splenic hypertrophy and

TABLE 3

0.05 CC. OF A TWO-LOOP EMULSION			0.1 CC. OF A TWO-LOOP EMULSION		
		cc. per cent			cc. per cent
1	Died 1 day after inoculation	$\begin{cases} 0.036 \\ 0.012 \end{cases}$	14	Killed 8 days after inoculation	$\begin{cases} 0.390 \\ 0.550 \end{cases} \quad 41$
2	Died 5 days after inoculation	$\begin{cases} 0.135 \\ 0.132 \end{cases}$	15	Killed 8 days after inoculation	$\begin{cases} 0.112 \\ 0.308 \end{cases} \quad 175$
3	Died 5 days after inoculation	$\begin{cases} 0.108 \\ 0.111 \end{cases} \quad 3$	16	Died 8 days after inoculation	$\begin{cases} 0.065 \\ 0.469 \end{cases} \quad 621$
4	Died 5 days after inoculation	$\begin{cases} 0.142 \\ 0.240 \end{cases} \quad 69$	17	Died 11 days after inoculation	$\begin{cases} 0.108 \\ 0.423 \end{cases} \quad 292$
5	Died 8 days after inoculation	$\begin{cases} 0.260 \\ 0.185 \end{cases}$	18	Died 12 days after inoculation	$\begin{cases} 0.060 \\ 0.441 \end{cases} \quad 635$
6	Killed 8 days after inoculation	$\begin{cases} 0.198 \\ 0.157 \end{cases}$			
7	Died 8 days after inoculation	$\begin{cases} 0.157 \\ 0.198 \end{cases} \quad 26$			
8	Killed 8 days after inoculation	$\begin{cases} 0.273 \\ 0.510 \end{cases} \quad 87$			
9	Died 8 days after inoculation	$\begin{cases} 0.148 \\ 0.329 \end{cases} \quad 122$			
10	Killed 8 days after inoculation	$\begin{cases} 0.072 \\ 0.305 \end{cases} \quad 324$			
11	Killed 13 days after inoculation	$\begin{cases} 0.127 \\ 0.252 \end{cases} \quad 98$			
12	Killed 13 days after inoculation	$\begin{cases} 0.127 \\ 0.287 \end{cases} \quad 126$			
13	Killed 13 days after inoculation	$\begin{cases} 0.209 \\ 0.564 \end{cases} \quad 170$			

Mice infected with the bacillus of mouse typhoid. The resulting disease causes splenic hypertrophy.

ulceration appears to be still a matter of uncertainty, mice with ulcerated tumors have been uniformly excluded from these experiments.

The ground having been thus cleared, it will be possible now to approach the main question: Is the spleen enlarged in mice immune to transplantable tumors, by virtue of their immunity?

EXPERIMENTS

In the following tables (4 to 11 inclusive) the mice have been numbered in agreement with the corresponding charts (figs. 1 to 9 inclusive). The upper row for each mouse represents the three dimensions of the spleen and its volume in cubic centimeters at the first measurement, while the lower gives similar data as collected at autopsy.

Figure 1 (experiment $\frac{63}{1281}$) reproduces 18 survivors of a group of thirty-six mice with spleens measured on March 16, 1916 (see table 4). Fifteen days later (March 31) these mice (Nos. 1 to 18) were immunized by the injection of 0.1 cc. of total embryo and placenta into the left axilla, and thirteen days after this immunizing treatment, they were inoculated with 0.02 cc. of carcinoma 63 in the opposite axilla.² They were killed, and their spleens were measured again on May 16—forty-six days after the immunizing injection. Reference to mice 19 to 28, normal untreated controls inoculated at the same time with an equal dose of the same tumor, will show that fourteen of the eighteen treated mice were immune to the tumor (Nos. 5 to 18). Yet of these fourteen immune mice, six, or 43 per cent, showed no hypertrophy of the spleen. Conversely, the mouse that developed the largest tumor (No. 1), and was therefore the least resistant of the entire group, had the third largest spleen in the series (341 per cent enlargement).

² The three propagable carcinomata used in these experiments (63, 206, and T) are from the Imperial Cancer Research Fund in London, whose Director, Dr. J. A. Murray, I wish to thank for his courtesy.

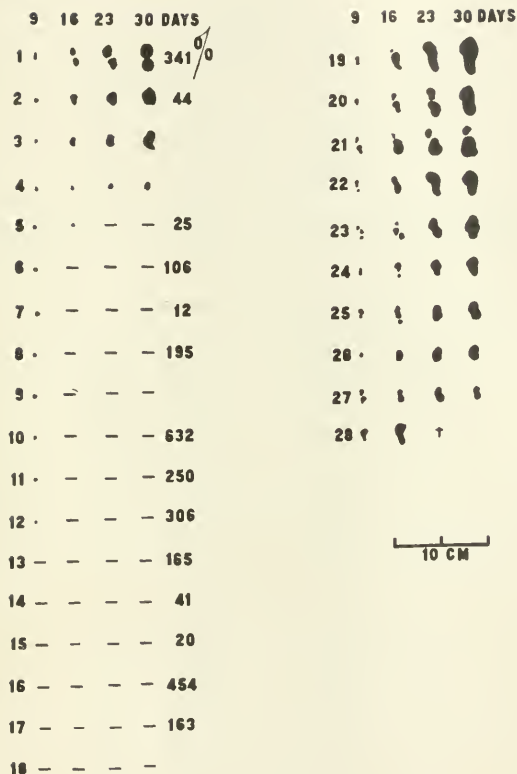


FIG. 1. EXPERIMENT $\frac{63}{1281}$

Mice 1 to 18 had their spleens measured March 16, 1916. Immunized March 31, with 0.1 cc. total embryo and placenta. Inoculated with 0.02 cc. of emulsified carcinoma 63 April 13. Killed and spleens measured May 16, sixty-one days after the first measurement (see table 4). The mice that proved immune did not all have enlarged spleens. Mice 19 to 28 are normal untreated controls.

TABLE 4
Experiment $\frac{63}{128 \text{ I}}$ (*fig. 1*)

	cc.	per cent
1	$\left\{ \begin{array}{l} 1.4 \times 0.35 \times 0.15 = 0.073 \\ 2.3 \times 0.7 \times 0.2 = 0.322 \end{array} \right\}$	341
2	$\left\{ \begin{array}{l} 1.6 \times 0.35 \times 0.15 = 0.084 \\ 1.8 \times 0.45 \times 0.15 = 0.121 \end{array} \right\}$	44
3	$\left\{ \begin{array}{l} 1.5 \times 0.45 \times 0.2 = 0.135 \\ 1.5 \times 0.5 \times 0.15 = 0.112 \end{array} \right\}$	
4	$\left\{ \begin{array}{l} 1.7 \times 0.4 \times 0.15 = 0.102 \\ 1.5 \times 0.4 \times 0.15 = 0.090 \end{array} \right\}$	
5	$\left\{ \begin{array}{l} 2.1 \times 0.45 \times 0.2 = 0.189 \\ 2.15 \times 0.55 \times 0.2 = 0.236 \end{array} \right\}$	25
6	$\left\{ \begin{array}{l} 1.6 \times 0.45 \times 0.2 = 0.144 \\ 1.7 \times 0.7 \times 0.25 = 0.297 \end{array} \right\}$	106
7	$\left\{ \begin{array}{l} 1.5 \times 0.4 \times 0.15 = 0.090 \\ 1.5 \times 0.45 \times 0.15 = 0.101 \end{array} \right\}$	12
8	$\left\{ \begin{array}{l} 1.3 \times 0.45 \times 0.15 = 0.088 \\ 2.0 \times 0.65 \times 0.2 = 0.260 \end{array} \right\}$	195
9	$\left\{ \begin{array}{l} 1.8 \times 0.45 \times 0.2 = 0.162 \\ 1.3 \times 0.35 \times 0.15 = 0.068 \end{array} \right\}$	
10	$\left\{ \begin{array}{l} 1.25 \times 0.35 \times 0.1 = 0.044 \\ 2.3 \times 0.7 \times 0.2 = 0.322 \end{array} \right\}$	632
11	$\left\{ \begin{array}{l} 1.4 \times 0.4 \times 0.15 = 0.084 \\ 2.1 \times 0.7 \times 0.2 = 0.294 \end{array} \right\}$	250
12	$\left\{ \begin{array}{l} 1.3 \times 0.35 \times 0.15 = 0.068 \\ 2.3 \times 0.6 \times 0.2 = 0.276 \end{array} \right\}$	306
13	$\left\{ \begin{array}{l} 2.0 \times 0.4 \times 0.15 = 0.120 \\ 2.65 \times 0.6 \times 0.2 = 0.318 \end{array} \right\}$	165
14	$\left\{ \begin{array}{l} 1.9 \times 0.6 \times 0.2 = 0.228 \\ 2.15 \times 0.75 \times 0.2 = 0.322 \end{array} \right\}$	41
15	$\left\{ \begin{array}{l} 1.6 \times 0.4 \times 0.15 = 0.096 \\ 1.7 \times 0.45 \times 0.15 = 0.115 \end{array} \right\}$	20

TABLE 4—*Concluded*

	<i>cc.</i>	<i>per cent</i>
16	$\left\{ \begin{array}{l} 1.5 \times 0.35 \times 0.1 = 0.052 \\ 2.4 \times 0.6 \times 0.2 = 0.288 \end{array} \right\}$	454
17	$\left\{ \begin{array}{l} 1.6 \times 0.4 \times 0.15 = 0.096 \\ 2.3 \times 0.55 \times 0.2 = 0.253 \end{array} \right\}$	163
18	$\left\{ \begin{array}{l} 1.6 \times 0.5 \times 0.2 = 0.160 \\ 1.7 \times 0.55 \times 0.15 = 0.140 \end{array} \right\}$	

No relation between splenic hypertrophy and immunity induced with normal mouse tissue.

TABLE 5

Experiment $\frac{63}{128 \text{ H}}$ (*fig. 2*)

	<i>cc.</i>	<i>per cent</i>
1	$\left\{ \begin{array}{l} 1.55 \times 0.45 \times 0.15 = 0.105 \\ 1.9 \times 0.55 \times 0.2 = 0.209 \end{array} \right\}$	99
2	$\left\{ \begin{array}{l} 1.55 \times 0.5 \times 0.2 = 0.155 \\ 1.75 \times 0.6 \times 0.15 = 0.157 \end{array} \right\}$	1
3	$\left\{ \begin{array}{l} 1.7 \times 0.4 \times 0.15 = 0.102 \\ 1.55 \times 0.4 \times 0.1 = 0.062 \end{array} \right\}$	
4	$\left\{ \begin{array}{l} 1.95 \times 0.6 \times 0.2 = 0.234 \\ 1.6 \times 0.45 \times 0.1 = 0.072 \end{array} \right\}$	
5	$\left\{ \begin{array}{l} 1.75 \times 0.55 \times 0.15 = 0.144 \\ 1.9 \times 0.5 \times 0.2 = 0.190 \end{array} \right\}$	32
6	$\left\{ \begin{array}{l} 1.3 \times 0.45 \times 0.15 = 0.088 \\ 1.3 \times 0.5 \times 0.15 = 0.097 \end{array} \right\}$	10
7	$\left\{ \begin{array}{l} 1.9 \times 0.55 \times 0.25 = 0.261 \\ 1.7 \times 0.55 \times 0.2 = 0.187 \end{array} \right\}$	
8	$\left\{ \begin{array}{l} 1.85 \times 0.5 \times 0.15 = 0.139 \\ 1.7 \times 0.5 \times 0.15 = 0.127 \end{array} \right\}$	
9	$\left\{ \begin{array}{l} 1.8 \times 0.5 \times 0.2 = 0.180 \\ 1.1 \times 0.35 \times 0.1 = 0.038 \end{array} \right\}$	
10	$\left\{ \begin{array}{l} 1.6 \times 0.45 \times 0.15 = 0.108 \\ 2.4 \times 0.7 \times 0.25 = 0.420 \end{array} \right\}$	289

TABLE 5—*Concluded*

	cc.	per cent
11	$\left\{ \begin{array}{l} 1.9 \times 0.5 \times 0.15 = 0.142 \\ 2.2 \times 0.6 \times 0.2 = 0.264 \end{array} \right\}$	86
12	$\left\{ \begin{array}{l} 1.9 \times 0.6 \times 0.25 = 0.285 \\ 1.85 \times 0.55 \times 0.2 = 0.203 \end{array} \right\}$	
13	$\left\{ \begin{array}{l} 1.55 \times 0.45 \times 0.1 = 0.070 \\ 1.5 \times 0.4 \times 0.1 = 0.060 \end{array} \right\}$	
14	$\left\{ \begin{array}{l} 1.55 \times 0.4 \times 0.1 = 0.062 \\ 2.15 \times 0.6 \times 0.15 = 0.193 \end{array} \right\}$	211
15	$\left\{ \begin{array}{l} 1.6 \times 0.4 \times 0.1 = 0.064 \\ 1.6 \times 0.4 \times 0.1 = 0.064 \end{array} \right\}$	
16	$\left\{ \begin{array}{l} 1.6 \times 0.4 \times 0.15 = 0.096 \\ 1.6 \times 0.45 \times 0.15 = 0.108 \end{array} \right\}$	12
17	$\left\{ \begin{array}{l} 1.5 \times 0.5 \times 0.2 = 0.150 \\ 1.7 \times 0.55 \times 0.15 = 0.140 \end{array} \right\}$	
18	$\left\{ \begin{array}{l} 2.2 \times 0.45 \times 0.2 = 0.198 \\ 2.4 \times 0.55 \times 0.15 = 0.198 \end{array} \right\}$	

No relation between splenic hypertrophy and immunity induced with spontaneous tumors.

TABLE 6

Experiment $\frac{63}{130}$ J (*fig. 3*)

	cc.	per cent
1	$\left\{ \begin{array}{l} 1.3 \times 0.4 \times 0.15 = 0.078 \\ 1.75 \times 0.6 \times 0.2 = 0.210 \end{array} \right\}$	170
2	$\left\{ \begin{array}{l} 1.4 \times 0.4 \times 0.15 = 0.084 \\ 2.0 \times 0.6 \times 0.2 = 0.240 \end{array} \right\}$	183
3	$\left\{ \begin{array}{l} 1.5 \times 0.4 \times 0.15 = 0.090 \\ 1.8 \times 0.6 \times 0.15 = 0.162 \end{array} \right\}$	80
4	$\left\{ \begin{array}{l} 1.6 \times 0.4 \times 0.15 = 0.096 \\ 1.85 \times 0.45 \times 0.15 = 0.125 \end{array} \right\}$	30
5	$\left\{ \begin{array}{l} 1.65 \times 0.5 \times 0.15 = 0.124 \\ 2.0 \times 0.55 \times 0.2 = 0.220 \end{array} \right\}$	77

TABLE 6—*Concluded*

	<i>cc.</i>	<i>per cent</i>
6	$\left\{ \begin{array}{l} 1.4 \times 0.45 \times 0.15 = 0.094 \\ 2.2 \times 0.6 \times 0.2 = 0.264 \end{array} \right\}$	181
7	$\left\{ \begin{array}{l} 1.25 \times 0.4 \times 0.1 = 0.050 \\ 2.15 \times 0.65 \times 0.2 = 0.279 \end{array} \right\}$	458
8	$\left\{ \begin{array}{l} 1.35 \times 0.45 \times 0.1 = 0.061 \\ 1.8 \times 0.45 \times 0.15 = 0.121 \end{array} \right\}$	98
9	$\left\{ \begin{array}{l} 1.35 \times 0.3 \times 0.15 = 0.061 \\ 1.8 \times 0.6 \times 0.15 = 0.162 \end{array} \right\}$	165
10	$\left\{ \begin{array}{l} 1.4 \times 0.5 \times 0.1 = 0.070 \\ 1.9 \times 0.6 \times 0.2 = 0.228 \end{array} \right\}$	226
11	$\left\{ \begin{array}{l} 1.75 \times 0.5 \times 0.15 = 0.131 \\ 1.9 \times 0.6 \times 0.2 = 0.228 \end{array} \right\}$	74
12	$\left\{ \begin{array}{l} 1.5 \times 0.35 \times 0.15 = 0.079 \\ 2.0 \times 0.45 \times 0.15 = 0.135 \end{array} \right\}$	71
13	$\left\{ \begin{array}{l} 1.25 \times 0.4 \times 0.1 = 0.050 \\ 1.8 \times 0.5 \times 0.15 = 0.135 \end{array} \right\}$	170
14	$\left\{ \begin{array}{l} 1.5 \times 0.45 \times 0.15 = 0.101 \\ 1.75 \times 0.5 \times 0.1 = 0.087 \end{array} \right\}$	
15	$\left\{ \begin{array}{l} 1.1 \times 0.4 \times 0.1 = 0.044 \\ 1.4 \times 0.5 \times 0.15 = 0.105 \end{array} \right\}$	139
16	$\left\{ \begin{array}{l} 1.2 \times 0.35 \times 0.1 = 0.042 \\ 1.9 \times 0.6 \times 0.2 = 0.228 \end{array} \right\}$	443
17	$\left\{ \begin{array}{l} 1.1 \times 0.3 \times 0.1 = 0.033 \\ 1.25 \times 0.4 \times 0.15 = 0.075 \end{array} \right\}$	127
18	$\left\{ \begin{array}{l} 1.4 \times 0.4 \times 0.15 = 0.084 \\ 2.1 \times 0.5 \times 0.15 = 0.157 \end{array} \right\}$	87
19	$\left\{ \begin{array}{l} 1.25 \times 0.3 \times 0.15 = 0.056 \\ 1.9 \times 0.4 \times 0.2 = 0.152 \end{array} \right\}$	171

No relation between splenic hypertrophy and immunity induced with spontaneous tumors. All spleens (except No. 11) less than 1.7 cm. in length at first measurement.

TABLE 7
 $\text{Experiment } \frac{T}{90 \text{ H}}$ (fig. 5)

	cc.	per cent
1	$\left\{ \begin{array}{l} 1.4 \times 0.4 \times 0.1 = 0.056 \\ 1.9 \times 0.35 \times 0.2 = 0.133 \end{array} \right\}$	137
2	$\left\{ \begin{array}{l} 1.4 \times 0.55 \times 0.15 = 0.115 \\ 1.8 \times 0.7 \times 0.15 = 0.189 \end{array} \right\}$	64
3	$\left\{ \begin{array}{l} 1.45 \times 0.5 \times 0.15 = 0.109 \\ 2.0 \times 0.6 \times 0.2 = 0.240 \end{array} \right\}$	120
4	$\left\{ \begin{array}{l} 1.3 \times 0.4 \times 0.1 = 0.052 \\ 1.9 \times 0.6 \times 0.2 = 0.228 \end{array} \right\}$	338
5	$\left\{ \begin{array}{l} 1.1 \times 0.35 \times 0.1 = 0.038 \\ 1.9 \times 0.5 \times 0.15 = 0.142 \end{array} \right\}$	273
6	$\left\{ \begin{array}{l} 1.3 \times 0.4 \times 0.1 = 0.052 \\ 1.75 \times 0.5 \times 0.2 = 0.175 \end{array} \right\}$	236
7	$\left\{ \begin{array}{l} 1.6 \times 0.5 \times 0.15 = 0.120 \\ 1.9 \times 0.5 \times 0.15 = 0.142 \end{array} \right\}$	180
8	$\left\{ \begin{array}{l} 1.5 \times 0.45 \times 0.15 = 0.101 \\ 2.1 \times 0.6 \times 0.2 = 0.252 \end{array} \right\}$	149
9	$\left\{ \begin{array}{l} 1.4 \times 0.4 \times 0.15 = 0.084 \\ 1.95 \times 0.65 \times 0.2 = 0.253 \end{array} \right\}$	200
10	$\left\{ \begin{array}{l} 1.6 \times 0.5 \times 0.2 = 0.160 \\ 2.1 \times 0.55 \times 0.2 = 0.231 \end{array} \right\}$	44
11	$\left\{ \begin{array}{l} 1.5 \times 0.5 \times 0.15 = 0.112 \\ 1.9 \times 0.6 \times 0.2 = 0.228 \end{array} \right\}$	103
12	$\left\{ \begin{array}{l} 1.4 \times 0.5 \times 0.1 = 0.070 \\ 1.7 \times 0.5 \times 0.15 = 0.127 \end{array} \right\}$	81
13	$\left\{ \begin{array}{l} 1.5 \times 0.45 \times 0.15 = 0.101 \\ 2.1 \times 0.6 \times 0.2 = 0.252 \end{array} \right\}$	149
14	$\left\{ \begin{array}{l} 1.6 \times 0.5 \times 0.15 = 0.120 \\ 2.1 \times 0.6 \times 0.2 = 0.252 \end{array} \right\}$	110
15	$\left\{ \begin{array}{l} 1.5 \times 0.4 \times 0.15 = 0.090 \\ 1.7 \times 0.45 \times 0.15 = 0.115 \end{array} \right\}$	28

TABLE 7—*Concluded*

	cc.	per cent
16	$\left\{ \begin{array}{l} 1.5 \times 0.4 \times 0.15 = 0.090 \\ 1.65 \times 0.45 \times 0.15 = 0.111 \end{array} \right\}$	23
17	$\left\{ \begin{array}{l} 1.6 \times 0.55 \times 0.2 = 0.176 \\ 1.9 \times 0.55 \times 0.15 = 0.157 \end{array} \right\}$	
18	$\left\{ \begin{array}{l} 1.5 \times 0.5 \times 0.15 = 0.112 \\ 1.75 \times 0.5 \times 0.15 = 0.131 \end{array} \right\}$	17
19	$\left\{ \begin{array}{l} 1.2 \times 0.35 \times 0.1 = 0.042 \\ 1.9 \times 0.55 \times 0.15 = 0.157 \end{array} \right\}$	273
20	$\left\{ \begin{array}{l} 1.4 \times 0.5 \times 0.2 = 0.140 \\ 2.25 \times 0.55 \times 0.2 = 0.247 \end{array} \right\}$	73
21	$\left\{ \begin{array}{l} 1.3 \times 0.4 \times 0.1 = 0.052 \\ 1.75 \times 0.4 \times 0.15 = 0.105 \end{array} \right\}$	102
22	$\left\{ \begin{array}{l} 1.55 \times 0.4 \times 0.15 = 0.093 \\ 2.1 \times 0.5 \times 0.15 = 0.157 \end{array} \right\}$	69
23	$\left\{ \begin{array}{l} 1.3 \times 0.4 \times 0.1 = 0.052 \\ 1.8 \times 0.5 \times 0.15 = 0.135 \end{array} \right\}$	159
24	$\left\{ \begin{array}{l} 1.0 \times 0.35 \times 0.1 = 0.035 \\ 1.9 \times 0.5 \times 0.2 = 0.190 \end{array} \right\}$	442
25	$\left\{ \begin{array}{l} 1.4 \times 0.45 \times 0.2 = 0.126 \\ 2.0 \times 0.5 \times 0.15 = 0.150 \end{array} \right\}$	19
26	$\left\{ \begin{array}{l} 1.4 \times 0.5 \times 0.15 = 0.105 \\ 1.9 \times 0.6 \times 0.2 = 0.228 \end{array} \right\}$	117
27	$\left\{ \begin{array}{l} 1.6 \times 0.5 \times 0.15 = 0.120 \\ 1.75 \times 0.45 \times 0.15 = 0.118 \end{array} \right\}$	
28	$\left\{ \begin{array}{l} 1.2 \times 0.45 \times 0.1 = 0.054 \\ 1.7 \times 0.7 \times 0.15 = 0.178 \end{array} \right\}$	229
29	$\left\{ \begin{array}{l} 1.3 \times 0.4 \times 0.1 = 0.052 \\ 1.8 \times 0.6 \times 0.15 = 0.162 \end{array} \right\}$	211
30	$\left\{ \begin{array}{l} 1.5 \times 0.4 \times 0.1 = 0.060 \\ 1.8 \times 0.5 \times 0.15 = 0.135 \end{array} \right\}$	125

Mice injected with non-immunizing material have enlarged spleens. All spleens under 1.7 cm. in length at first measurement.

TABLE 8
Experiment $\frac{63}{134 \text{ T}}$ (fig. 6)

	<i>cc.</i>	<i>per cent</i>
1	$\left\{ \begin{array}{l} 1.35 \times 0.35 \times 0.15 = 0.071 \\ 1.8 \times 0.45 \times 0.15 = 0.121 \end{array} \right\}$	70
2	$\left\{ \begin{array}{l} 1.2 \times 0.4 \times 0.15 = 0.072 \\ 1.7 \times 0.5 \times 0.15 = 0.127 \end{array} \right\}$	76
3	$\left\{ \begin{array}{l} 1.5 \times 0.45 \times 0.15 = 0.101 \\ 1.9 \times 0.5 \times 0.15 = 0.142 \end{array} \right\}$	41
4	$\left\{ \begin{array}{l} 1.2 \times 0.3 \times 0.1 = 0.036 \\ 1.75 \times 0.5 \times 0.15 = 0.131 \end{array} \right\}$	264
5	$\left\{ \begin{array}{l} 1.4 \times 0.4 \times 0.15 = 0.084 \\ 2.1 \times 0.6 \times 0.2 = 0.252 \end{array} \right\}$	200
6	$\left\{ \begin{array}{l} 1.3 \times 0.45 \times 0.15 = 0.088 \\ 1.7 \times 0.5 \times 0.15 = 0.127 \end{array} \right\}$	44
7	$\left\{ \begin{array}{l} 1.3 \times 0.4 \times 0.1 = 0.052 \\ 1.9 \times 0.5 \times 0.2 = 0.190 \end{array} \right\}$	265
8	$\left\{ \begin{array}{l} 1.2 \times 0.4 \times 0.1 = 0.048 \\ 1.8 \times 0.5 \times 0.15 = 0.135 \end{array} \right\}$	181
9	$\left\{ \begin{array}{l} 1.25 \times 0.4 \times 0.1 = 0.050 \\ 1.5 \times 0.4 \times 0.15 = 0.090 \end{array} \right\}$	80
10	$\left\{ \begin{array}{l} 1.3 \times 0.4 \times 0.1 = 0.052 \\ 1.85 \times 0.55 \times 0.2 = 0.203 \end{array} \right\}$	290
11	$\left\{ \begin{array}{l} 1.5 \times 0.5 \times 0.15 = 0.112 \\ 1.5 \times 0.4 \times 0.15 = 0.090 \end{array} \right\}$	
12	$\left\{ \begin{array}{l} 1.2 \times 0.4 \times 0.15 = 0.072 \\ 2.2 \times 0.7 \times 0.3 = 0.462 \end{array} \right\}$	542
13	$\left\{ \begin{array}{l} 1.25 \times 0.4 \times 0.1 = 0.050 \\ 1.6 \times 0.5 \times 0.15 = 0.120 \end{array} \right\}$	140
14	$\left\{ \begin{array}{l} 1.25 \times 0.4 \times 0.1 = 0.050 \\ 1.7 \times 0.5 \times 0.15 = 0.127 \end{array} \right\}$	154
15	$\left\{ \begin{array}{l} 1.4 \times 0.5 \times 0.15 = 0.105 \\ 2.3 \times 0.6 \times 0.2 = 0.276 \end{array} \right\}$	163

TABLE 8—*Concluded*

	cc.	per cent
16	$\left\{ \begin{array}{l} 1.3 \times 0.4 \times 0.15 = 0.078 \\ 1.9 \times 0.6 \times 0.2 = 0.228 \end{array} \right\}$	192
17	$\left\{ \begin{array}{l} 1.2 \times 0.35 \times 0.1 = 0.042 \\ 1.7 \times 0.5 \times 0.2 = 0.170 \end{array} \right\}$	305
18	$\left\{ \begin{array}{l} 1.1 \times 0.3 \times 0.1 = 0.033 \\ 1.6 \times 0.5 \times 0.15 = 0.120 \end{array} \right\}$	264
19	$\left\{ \begin{array}{l} 1.3 \times 0.4 \times 0.1 = 0.052 \\ 2.0 \times 0.6 \times 0.2 = 0.240 \end{array} \right\}$	361
20	$\left\{ \begin{array}{l} 1.4 \times 0.4 \times 0.15 = 0.084 \\ 1.6 \times 0.45 \times 0.15 = 0.108 \end{array} \right\}$	28
21	$\left\{ \begin{array}{l} 1.5 \times 0.4 \times 0.15 = 0.090 \\ 1.7 \times 0.5 \times 0.15 = 0.127 \end{array} \right\}$	41
22	$\left\{ \begin{array}{l} 1.3 \times 0.45 \times 0.15 = 0.088 \\ 1.7 \times 0.5 \times 0.15 = 0.127 \end{array} \right\}$	44
23	$\left\{ \begin{array}{l} 1.3 \times 0.4 \times 0.1 = 0.052 \\ 1.8 \times 0.5 \times 0.2 = 0.180 \end{array} \right\}$	246
24	$\left\{ \begin{array}{l} 1.5 \times 0.4 \times 0.15 = 0.090 \\ 2.2 \times 0.6 \times 0.2 = 0.264 \end{array} \right\}$	193
25	$\left\{ \begin{array}{l} 1.3 \times 0.4 \times 0.1 = 0.052 \\ 1.9 \times 0.65 \times 0.2 = 0.247 \end{array} \right\}$	375
26	$\left\{ \begin{array}{l} 1.35 \times 0.4 \times 0.15 = 0.081 \\ 1.8 \times 0.5 \times 0.15 = 0.135 \end{array} \right\}$	67

Mice injected with non-immunizing material have enlarged spleens. All spleens under 1.7 cm. in length at first measurement.

TABLE 9
Experiment $\frac{206}{177 \text{ L}}$ and $\frac{206}{178 \text{ J}}$ (fig. 7)

	cc.	per cent
1	$\left\{ \begin{array}{l} 1.1 \times 0.35 \times 0.05 = 0.019 \\ 2.0 \times 0.6 \times 0.2 = 0.240 \end{array} \right\}$	1163
2	$\left\{ \begin{array}{l} 1.6 \times 0.5 \times 0.15 = 0.120 \\ 1.7 \times 0.6 \times 0.15 = 0.153 \end{array} \right\}$	27
3	$\left\{ \begin{array}{l} 2.0 \times 0.6 \times 0.2 = 0.240 \\ 2.0 \times 0.75 \times 0.2 = 0.300 \end{array} \right\}$	25
4	$\left\{ \begin{array}{l} 2.0 \times 0.6 \times 0.2 = 0.240 \\ 1.7 \times 0.5 \times 0.15 = 0.127 \end{array} \right\}$	
5	$\left\{ \begin{array}{l} 1.7 \times 0.5 \times 0.2 = 0.170 \\ 1.7 \times 0.55 \times 0.15 = 0.140 \end{array} \right\}$	
6	$\left\{ \begin{array}{l} 1.4 \times 0.5 \times 0.15 = 0.105 \\ 1.6 \times 0.55 \times 0.15 = 0.132 \end{array} \right\}$	26
7	$\left\{ \begin{array}{l} 2.0 \times 0.5 \times 0.15 = 0.150 \\ 2.0 \times 0.65 \times 0.2 = 0.260 \end{array} \right\}$	73
8	$\left\{ \begin{array}{l} 1.5 \times 0.35 \times 0.15 = 0.079 \\ 2.0 \times 0.5 \times 0.15 = 0.150 \end{array} \right\}$	90
9	$\left\{ \begin{array}{l} 1.6 \times 0.5 \times 0.15 = 0.120 \\ 1.8 \times 0.6 \times 0.2 = 0.216 \end{array} \right\}$	80
10	$\left\{ \begin{array}{l} 1.7 \times 0.55 \times 0.15 = 0.140 \\ 1.7 \times 0.5 \times 0.15 = 0.127 \end{array} \right\}$	
11	$\left\{ \begin{array}{l} 1.3 \times 0.5 \times 0.1 = 0.065 \\ 1.5 \times 0.5 \times 0.15 = 0.112 \end{array} \right\}$	72
12	$\left\{ \begin{array}{l} 2.0 \times 0.6 \times 0.2 = 0.240 \\ 2.0 \times 0.65 \times 0.2 = 0.260 \end{array} \right\}$	8
13	$\left\{ \begin{array}{l} 1.35 \times 0.4 \times 0.1 = 0.054 \\ 1.7 \times 0.5 \times 0.15 = 0.127 \end{array} \right\}$	135
14	$\left\{ \begin{array}{l} 1.6 \times 0.5 \times 0.15 = 0.120 \\ 1.6 \times 0.5 \times 0.15 = 0.120 \end{array} \right\}$	
15	$\left\{ \begin{array}{l} 1.9 \times 0.55 \times 0.15 = 0.157 \\ 1.9 \times 0.6 \times 0.2 = 0.228 \end{array} \right\}$	45

TABLE 9—*Concluded*

		cc.	per cent
16	$\left\{ \begin{array}{l} 1.3 \times 0.5 \times 0.15 = 0.097 \\ 1.5 \times 0.6 \times 0.2 = 0.180 \end{array} \right\}$		85
17	$\left\{ \begin{array}{l} 2.0 \times 0.6 \times 0.2 = 0.240 \\ 2.1 \times 0.55 \times 0.2 = 0.231 \end{array} \right\}$		
18	$\left\{ \begin{array}{l} 1.9 \times 0.6 \times 0.2 = 0.228 \\ 1.4 \times 0.4 \times 0.15 = 0.084 \end{array} \right\}$		
19	$\left\{ \begin{array}{l} 1.9 \times 0.65 \times 0.2 = 0.247 \\ 1.75 \times 0.45 \times 0.15 = 0.118 \end{array} \right\}$		
20	$\left\{ \begin{array}{l} 1.75 \times 0.5 \times 0.15 = 0.131 \\ 1.7 \times 0.45 \times 0.15 = 0.115 \end{array} \right\}$		
21	$\left\{ \begin{array}{l} 1.3 \times 0.5 \times 0.15 = 0.097 \\ 1.8 \times 0.6 \times 0.2 = 0.216 \end{array} \right\}$		123
22	$\left\{ \begin{array}{l} 1.7 \times 0.5 \times 0.2 = 0.170 \\ 1.8 \times 0.55 \times 0.2 = 0.198 \end{array} \right\}$		16

No relation between splenic hypertrophy and immunity induced with transplantable tumors.

TABLE 10
Experiment $\frac{T}{89.1}$ (*fig. 8*)

		cc.
1.....	1.7 $\times$ 0.5 $\times$ 0.2	= 0.170
2.....	1.7 $\times$ 0.5 $\times$ 0.2	= 0.170
3.....	1.8 $\times$ 0.5 $\times$ 0.15	= 0.135
4.....	2.4 $\times$ 0.55 $\times$ 0.2	= 0.264
5.....	1.8 $\times$ 0.5 $\times$ 0.2	= 0.180
6.....	1.75 $\times$ 0.6 $\times$ 0.2	= 0.210
7.....	1.7 $\times$ 0.55 $\times$ 0.2	= 0.187
8.....	2.4 $\times$ 0.8 $\times$ 0.25	= 0.480
9.....	1.8 $\times$ 0.6 $\times$ 0.2	= 0.216
10.....	2.1 $\times$ 0.7 $\times$ 0.3	= 0.441
11.....	1.8 $\times$ 0.5 $\times$ 0.2	= 0.180
12.....	2.0 $\times$ 0.75 $\times$ 0.25	= 0.375
13.....	1.8 $\times$ 0.55 $\times$ 0.2	= 0.198
14.....	1.75 $\times$ 0.5 $\times$ 0.2	= 0.175
15.....	2.0 $\times$ 0.8 $\times$ 0.2	= 0.320
16.....	2.2 $\times$ 0.7 $\times$ 0.25	= 0.385
17.....	1.8 $\times$ 0.6 $\times$ 0.2	= 0.216
18.....	2.1 $\times$ 0.7 $\times$ 0.2	= 0.294
19.....	1.9 $\times$ 0.5 $\times$ 0.3	= 0.285
20.....	1.5 $\times$ 0.6 $\times$ 0.2	= 0.180

No relation between splenic hypertrophy and natural immunity.

TABLE 11.
Experiment $\frac{63}{134 Q}$ (fig. 9)

				cc.
1.....	2.3	$\times 0.65$	$\times 0.25$	= 0.374
2.....	2.1	$\times 0.65$	$\times 0.2$	= 0.273
3.....	1.7	$\times 0.7$	$\times 0.2$	= 0.238
4.....	2.1	$\times 0.6$	$\times 0.2$	= 0.252
5.....	1.6	$\times 0.6$	$\times 0.2$	= 0.192
6.....	1.75	$\times 0.6$	$\times 0.2$	= 0.210
7.....	1.7	$\times 0.5$	$\times 0.2$	= 0.170
8.....	1.55	$\times 0.55$	$\times 0.25$	= 0.213
9.....	2.1	$\times 0.6$	$\times 0.2$	= 0.252
10.....	2.2	$\times 0.7$	$\times 0.25$	= 0.385
11.....	1.7	$\times 0.55$	$\times 0.15$	= 0.140
12.....	1.7	$\times 0.5$	$\times 0.15$	= 0.127
13.....	2.0	$\times 0.6$	$\times 0.2$	= 0.240
14.....	1.7	$\times 0.6$	$\times 0.15$	= 0.153
15.....	1.8	$\times 0.5$	$\times 0.2$	= 0.180
16.....	1.75	$\times 0.45$	$\times 0.2$	= 0.157
17.....	1.8	$\times 0.6$	$\times 0.2$	= 0.216
18.....	1.9	$\times 0.55$	$\times 0.15$	= 0.157
19.....	2.0	$\times 0.6$	$\times 0.25$	= 0.300
20.....	2.2	$\times 0.65$	$\times 0.2$	= 0.286

No relation between splenic hypertrophy and natural immunity.

In another experiment ($\frac{63}{128 H}$, fig. 2) spontaneous tumors were the immunizing material employed. The spleens of thirty-six mice were measured (see table 5) on March 14, 1916, and fifteen days afterward (March 29) 0.05 cc. of a mixed emulsion of seven spontaneous tumors was deposited in the left axilla. Fifteen days following immunization (April 13) 0.02 cc. of carcinoma 63 was inoculated in the right axilla, and the survivors (mice 1 to 18) were killed and their spleens measured thirty-four days after immunization (May 2). While the degree of immunity was not so high as in the first experiment, seven of the eighteen (Nos. 12 to 18) were undoubtedly immune. Yet in six (86 per cent) of these seven refractory animals the spleen had not enlarged. Mice 19 to 28 are normal untreated controls.

Since it was not known whether a period of from thirty-four to forty-six days suffices for the spleen to enlarge, the experiment was repeated ($\frac{63}{130 J}$, fig. 3) with a longer interval between



FIG. 2. EXPERIMENT $\frac{63}{128 H}$

Mice 1 to 18 had their spleens measured March 14, 1916. Immunized March 29 with 0.05 cc. of emulsified spontaneous tumors. None of the inoculated material grew. Inoculated with 0.02 cc. of emulsified carcinoma 63 April 13. Killed and spleens measured May 2, forty-nine days after the first measurement (see table 5). The mice that proved immune did not all have enlarged spleens. Mice 19 to 28 are normal untreated controls.

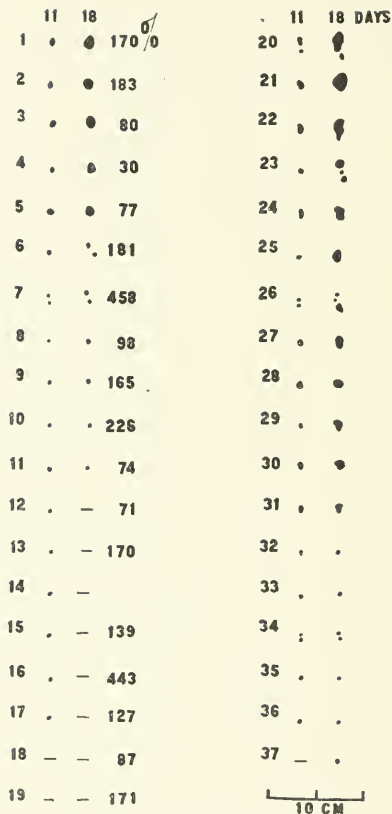


FIG. 3. EXPERIMENT ⁶³_{130 J}

Mice 1 to 19 had their spleens measured June 8, 1916. Injected June 15 with 0.05 cc. of emulsified spontaneous tumors. None of the inoculated material grew. Inoculated with 0.003 gm. of emulsified carcinoma 63 July 27. Killed and spleens measured August 18, seventy-one days after the first measurement (see table 6). Many of the spleens are enlarged, but the interval between the two measurements was long (see fig. 4). Mice 20 to 37 are normal untreated controls.

immunization and final measurement. On June 8, 1916, the spleens of thirty-six mice were measured (see table 6) and on June 15, seven days later, the animals were injected in the left axilla with a mixed emulsion of seven spontaneous tumors; no growths resulted from this preliminary treatment. Forty-two days after this (on July 27) the survivors (mice 1 to 19) were inoculated on the opposite side with 0.003 gram of carcinoma 63 by the needle method, and on August 18, sixty-four days following immunization, the survivors (Nos. 1 to 19) were killed and their spleens were measured. Mice 20 to 37 are normal untreated controls.

In this experiment an additional source of error has been eliminated, only mice with spleens less than 1.7 cm. in length having been employed (except that in No. 11 the spleen was 1.75 cm. long), so that this group contains only animals in which the organ approximated its normal size. The objection that in experiments 1 and 2 the spleen had already reached the limit of its ability to enlarge, and that an hypertrophy that might otherwise have taken place has thereby been masked, is here fully answered.

Eight of the treated mice (Nos. 12 to 19) were immune. In all but one of the eight (No. 14) the spleen had undergone an increase of more than 50 per cent of its original size; but it is to be remembered that these animals had been exposed to infection with mouse typhus for a longer period than those in the two experiments preceding. This, and not termination of the two preceding experiments before sufficient time had been given for the organ to enlarge, probably explains the high percentage of enlarged spleens in the immune mice of figure 3; for it will be immediately noted that among the whole group of nineteen mice, irrespective of whether or not tumors developed, only two mice escaped splenic hypertrophy.

The fact that enlargement of the spleen is a function of the length of time that a mouse has been kept in a cage with other mice, is well brought out by figure 4, which represents graphically the percentage of animals with enlarged spleens in the eight experiments discussed in this paper; the time is the number of days between the first and final measurement of the spleen.

The curve, fairly regular for a biological experiment, shows that irrespective of whether or not he is immune to propagable neoplasms, the longer a mouse is exposed to mouse typhoid the greater is the chance that his spleen will undergo hypertrophy. Thus 20 per cent of the mice kept together in a cage for fourteen days developed splenic hypertrophy, whereas from 77 to 89 per cent of those exposed for more than seventy days had enlarged spleens. Between these extremes the curve is comparatively uniform, except for a break at one point which cannot be explained. The most obvious way to account for it is to assume that this group was a healthier lot of mice; yet the number that died before the completion of the experiment was no less than in other experiments. The data from which the curve was drawn may be summarized as follows:

		TABLE	DAYS	PER CENT
No tumor..... <u>206</u> and <u>206</u>	No preliminary treatment	1	14	20
<u>177</u> L <u>178</u> J	Immunized with tumor	9	39	39
No tumor..... <u>63</u>	No preliminary treatment	2	47	47
<u>128</u> H	Immunized with tumor	5	49	22
<u>63</u>				
<u>128</u> I	Immunized with embryo.....	4	61	50
<u>63</u>				
<u>130</u> J	Immunized with tumor	6	71	89
<u>63</u>				
<u>134</u> T	Injected with boiled embryo	8	78	77
<u>T</u>				
<u>90</u> H	Injected with boiled tumor	7	126	77

The conclusion that the splenic enlargement in the immune mice of the preceding experiment is not due to their immunity is supported by the results of the next two experiments, in which the situation is analyzed still further. Boiled mouse tissue, which is incapable of producing resistance to transplantable tumors, was inoculated and the mice were set aside for a long period as before.

In experiment $\frac{T}{90H}$, figure 5, the spleens of thirty-six mice were measured on October 31, 1916, and all those with spleens

exceeding 1.7 cm. in length were rejected (table 7). Into the left axilla of these animals there was introduced, six days later, 0.1 cc. of a mixed emulsion of three spontaneous tumors that had been boiled for ten minutes. On January 3, 1917, sixty-four days following preliminary measurement, the thirty survivors were inoculated in the right axilla with 0.003 gram of carcinoma T by the needle method. The outcome of this inoculation (mice 1 to 30) was similar to that in untreated controls (mice 31 to 50) inoculated at the same time and with fragments of the same tumor.

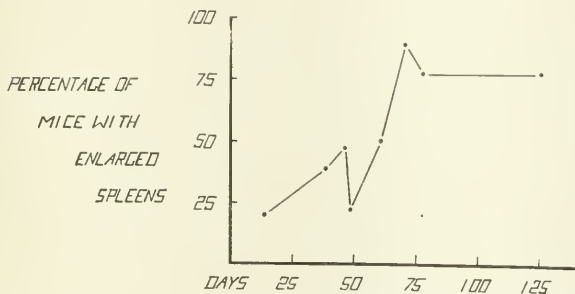
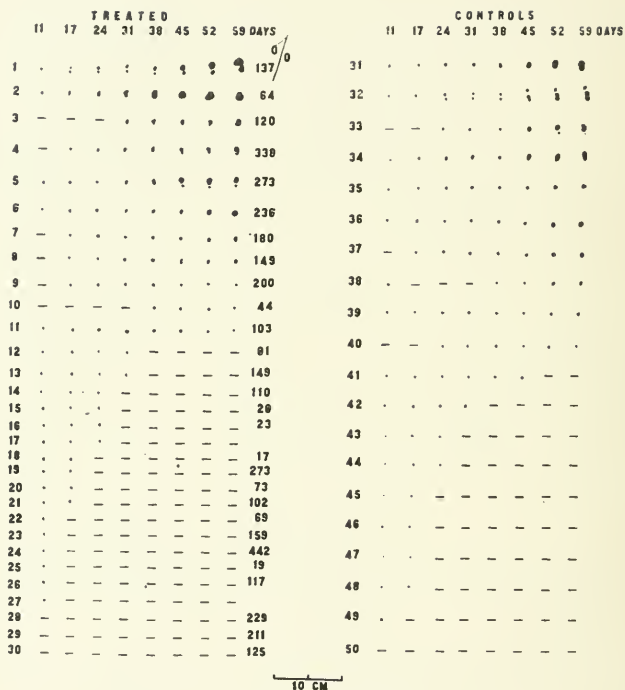


FIG. 4. The curve shows that the longer mice are exposed to mouse typhoid by being kept together in a cage, the greater is the chance that they will develop splenic hypertrophy.

Although the tumor grew in mice 1 to 8 fully as well as it did in the untreated controls, the spleen in all these eight mice was found enlarged more than 50 per cent of its original size at autopsy March 6, 1917. And the only mice in which there is less than 50 per cent increase in volume are to be found among Nos. 9 to 30, in which the tumor grew poorly, or not at all.

A similar experiment ($^{63}_{134}\text{T}$) is reproduced in figure 6 (table 8). On October 30, 1916, the spleens of thirty-six mice were measured; the series includes only those animals in which this organ was 1.7 cm. or less in length. Two days later (November 1) the mice were injected in the right axilla with 0.1 cc. of an

FIG. 5. EXPERIMENT $\frac{T}{90H}$

Mice 1 to 30 had their spleens measured October 31, 1916. Injected November 6 with 0.1 cc. of a spontaneous tumor emulsion that had been boiled for ten minutes. Inoculated January 3, 1917 with 0.003 gm. carcinoma T. Killed and spleens measured March 6, 1917, 126 days after the first measurement (see table 7). Many of the spleens are enlarged, but the interval between the two measurements was long (see fig. 4). Mice 31 to 50 are normal untreated controls.

TREATED				CONTROLS				
	9	18	25 DAYS		9	18	25 DAYS	
1	•	•	●	70	27	•	•	●
2	•	•	●	76	28	•	•	●
3	•	•	•	41	29	•	•	•
4	•	•	•	264	30	•	•	•
5	•	•	•	200	31	•	•	•
6	•	•	•	44	32	•	•	•
7	•	•	•	265	33	•	•	•
8	•	•	•	181	34	•	•	•
9	•	•	•	80	35	•	•	•
10	•	•	•	290	36	•	•	•
11	•	•	•		37	•	•	•
12	•	•	•	542	38	•	•	•
13	•	•	•	140	39	•	•	•
14	•	•	•	154	40	—	•	•
15	•	•	—	163	41	•	•	•
16	•	•	—	192	42	•	•	•
17	•	•	—	305	43	•	•	•
18	•	—	—	264	44	•	•	•
19	•	—	—	361	45	•	•	•
20	—	—	—	28	46	•	•	•
21	—	—	—	41	47	•	•	•
22	—	—	—	44	48	•	•	•
23	—	—	—	246	49	•	•	—
24	—	—	—	193	50	•	•	—
25	—	—	—	375	51	•	—	—
26	—	—	—	67	52	—	—	—
					53	—	—	—
					54	—	—	—
					55	—	—	—
					56	—	—	—
					57	—	—	—

10 CM

10 CM

FIG. 6. EXPERIMENT $\frac{63}{134T}$

Mice 1 to 26 had their spleens measured October 30, 1916. Injected November 1 with 0.1 cc. of total embryo and placenta boiled for ten minutes. Inoculated December 21 with 0.002 gm. carcinoma 63. Killed and spleens measured January 16, 1917, seventy-eight days after the first measurement (see table 8). Although boiled tissues do not immunize, many of the spleens are enlarged. Mice 27-57 are normal untreated controls.

emulsion of total mouse embryo and placenta that had been boiled for ten minutes, and fifty days after this treatment the absence of immunity was demonstrated by inoculation into the right axilla of 0.002 gram of carcinoma 63 by the needle method. The mice were killed and their spleens were measured on January 16, 1917—seventy-six days after treatment with the boiled material. The outcome of the tumor inoculation is about the same in the surviving mice of the treated series (1 to 26) as it is in the normal controls (27 to 57). As in the controls, a number of the treated mice were more or less refractory, but there seems to be no relation between the resistance and the size of the spleen. The absence of more than 50 per cent increase in mouse 3, and the slight excess over this figure in mice 1 and 2, the three mice with the largest tumors, might appear at first glance to be suggestive, until it is noticed that in three of those that were entirely resistant (Nos. 20, 21, and 22) the spleen showed no enlargement above the personal error, and that in a fourth (No. 26) the increase was slight. In mouse 11, again, whose tumor remained stationary, the spleen was smaller than at the first measurement.

It cannot be objected against the two preceding experiments that the spleens which were enlarged had increased in size because tumors T and 63 during their proliferation had immunized the mice, for Russell (33) has shown that both these neoplasms belong to a type of new growth which does not immunize the host during its sojourn.

Since the animals had received no immunizing treatment, and since their tumors had not rendered them refractory, there is no explanation for their splenic hypertrophy except: (a) infection of one sort or another (probably with the bacillus of mouse typhoid); or (b) natural immunity. The latter alternative will be taken up presently, and shown to be inadequate.

In the meantime, it is necessary to meet a really valid objection which may be raised against the preceding experiments: That the resistance engendered by the introduction and absorption of living tissue, as in the three first experiments, is different from that evoked by a growing tumor, and that it may not be

accompanied by splenic hypertrophy. It is fortunate that the work of Russell previously referred to has given us another method of producing immunity at will. This author has proved that although certain transplantable neoplasms do not immunize the host during their residence therein, certain others will produce a highly efficient resistance, so that reinoculation with these or other strains will be unsuccessful. Such a neoplasm is carcinoma 206, which will immunize practically every mouse in which it has grown for about two weeks, as Russell has so well shown. This tumor was therefore employed as follows (experiment $\frac{206}{177L}$ and $\frac{206}{178J}$, fig. 7). The spleens of a group of thirty-six mice were measured (table 9) on March 23, 1916, and thirteen days later (April 5) these mice were inoculated in the right axilla with 0.02 cc. of an emulsion of carcinoma 206. In order to prove that they were immune, they were inoculated twelve days later in the opposite axilla with a similar dose of the same tumor; twelve normal untreated mice (Nos. 23 to 34) were inoculated at the same time and with the same emulsion as a control to the reinoculation. In figure 7, Nos. 1 to 22 are the surviving animals with measured spleens, and the tumors to the left of the black line are those resulting from the first inoculation, while the dashes to the right of the line show the outcome of reinoculation. All were immune. The controls, mice 23 to 34, show that the material used for the second inoculation was able to grow in nearly half of the twelve normal mice inoculated; hence it should have grown also in about the same proportion of mice 1 to 22, had these not been made refractory by the first implantation. It would be difficult to devise a more exact experiment than this in cancer research, for here one has twenty-two mice with spleens of known size, and definitely proved to be immune, for the negative results of the reinoculation were corroborated at autopsy (May 1, 1916). Yet in fourteen of the twenty-two (64 per cent) the spleen has not increased 50 per cent in volume; in one of these it was the same size, and in seven actually smaller.

It was suggested in a preceding paragraph that certain mice may have had enlarged spleens because they were naturally

immune, though such an hypothesis loses much of its value when it is recollected that many mice with actively growing tumors have large spleens. And the assumption is still further undermined by the demonstration (figs. 8 and 9) that mice with splenic hypertrophy are not resistant to tumors. These figures reproduce the results following inoculation of two different mouse carcinomata into mice with enlarged spleens (tables 10 and 11). The outcome, on the left-hand side of each figure, is to be compared with growth of the same tumor, inoculated at the same time and in similar amount, into mice with spleens of approximately normal volume, on the right-hand side. Splenic hypertrophy evidently confers not the slightest resistance.

It is admitted, however, assuming for the moment that the refractory state does increase the volume of the spleen, that other causes might enlarge it as well; indeed, it has been proved that they actually do. Therefore, even though immunity does bring about hypertrophy, a mouse might have an enlarged spleen and still not be refractory to tumor inoculation, because the hypertrophy had been produced by some unrelated factor. But if immunity and splenic enlargement do go hand in hand, it may well be supposed that the mouse with the largest spleen will be most highly immune. Table 10 does not support this view. The two largest spleens (0.480 cc. and 0.441 cc.) belong to mice 8 and 10 (fig. 8), in which the tumor grew progressively. And while it is true that mice 1, 2, and 3, which developed large growths, had the smallest spleens in the series (0.170 cc., 0.170 cc., and 0.135 cc.), it is also true that mice 4, 5, and 9, with tumors

FIG. 7. EXPERIMENT $\frac{206}{177L}$ AND $\frac{206}{178J}$

Mice 1 to 22 had their spleens measured March 23, 1916. Immunized by the growth of carcinoma 206, 0.02 cc. of which was inoculated as an emulsion April 5. The results of this inoculation appear to the left of the vertical line. On April 17 the immunity of these mice was proved by inoculation of 0.02 cc. of an emulsion of the same tumor into the opposite axilla. The negative outcome of this inoculation twelve days later is shown to the right of the vertical line. The mice were killed and their spleens measured May 1, 39 days after the first measurement (see table 9). There is no relation between immunity and splenic hypertrophy. Mice 23 to 34 are normal untreated controls to the second inoculation.

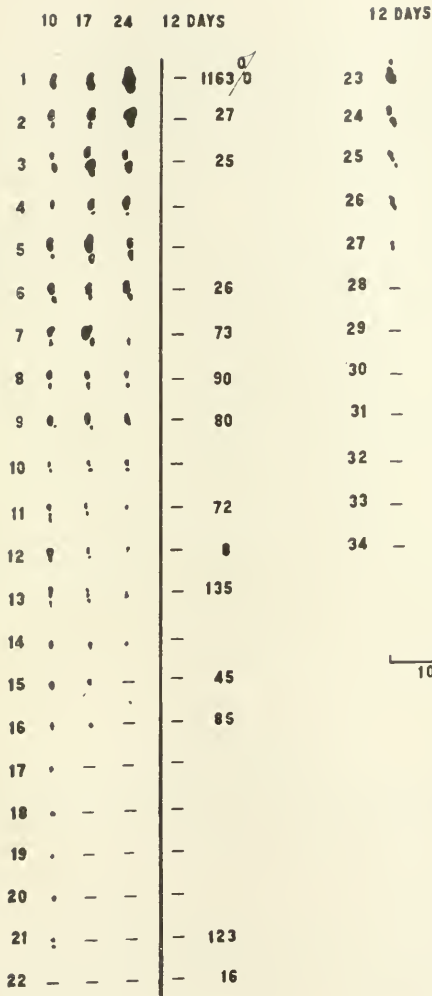


FIG. 7

almost equally large, had spleens (0.264 cc., 0.180 cc., and 0.216 cc.) no smaller than many of the immune animals. The average size of the spleens of the ten mice with progressively growing tumors is 0.245 cc., and of the ten immunes, 0.261 cc.; the latter exceeds the former by only 0.016 cc., or 6 per cent—a negligible

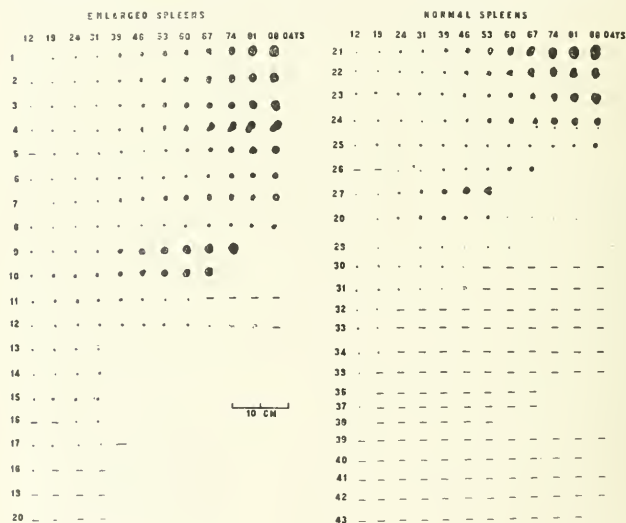


FIG. 8. EXPERIMENT $\frac{T}{89I}$

Mice 1 to 20 had enlarged spleens (see table 10). Mice 21 to 43 had spleens of approximately normal size (1.7 cm. or less in length). Mice with splenic hypertrophy are not resistant to tumor inoculation.

figure compared with the 50 per cent which has to be allowed for personal error.

Table 11 shows even more definitely the lack of any relation between immunity and the size of the spleen. In mice 1 to 12 (fig. 9) the tumors grew progressively and more or less rapidly.

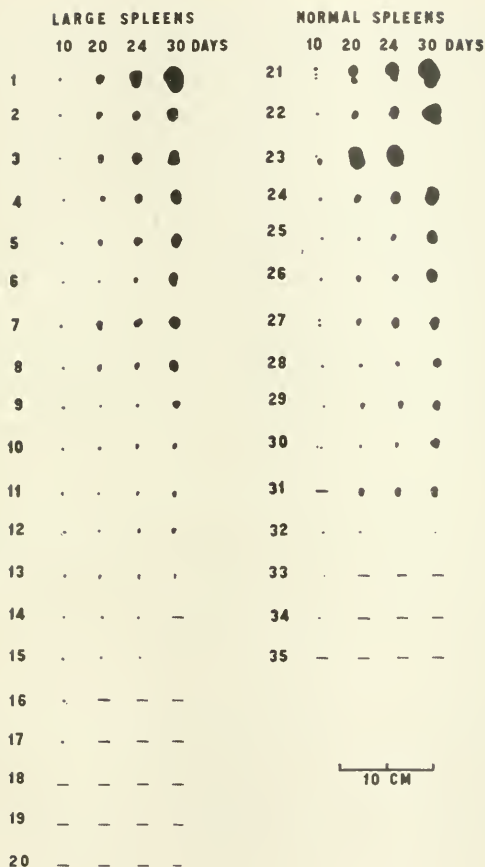


FIG. 9. EXPERIMENT $\frac{63}{134 Q}$

Mice 1 to 20 had enlarged spleens and mice 21 to 35 spleens of approximately normal size (see table 11). Mice with splenic hypertrophy are not resistant to tumor inoculation.

Here again the two largest spleens (0.385 cc. and 0.374 cc.) are found in mice (Nos. 10 and 1) with progressively growing tumors. The two next largest (0.300 cc. and 0.286 cc.) are in resistant animals (mice 19 and 20), but the three next largest (0.273 cc., 0.252 cc., and 0.252 cc.) are in mice with progressively growing neoplasms (Nos. 2, 4, and 9). And though the three smallest spleens (0.127 cc., 0.140 cc., and 0.170 cc.) are found in mice with progressively growing tumors (Nos. 12, 11, and 7), three almost equally small (0.153 cc., 0.157 cc., and 0.157 cc.) occur among the immunes (Nos. 14, 16, and 18). The average size of the spleens in the mice with progressively growing tumors is 0.235 cc., while in the immunes it is somewhat less—0.211 cc. The difference, 0.024 cc. (or 11 per cent) is closely equivalent to that in the preceding experiment, except that the larger spleens are found now, not in the resistant, but in the susceptible mice. This difference, however, like the first, may be disregarded, being far below the personal error.

DISCUSSION

The two experiments taken together show that the presence or absence of an enlarged spleen in natural, as in acquired immunity, is a matter of pure chance so far as susceptibility or resistance to tumors is concerned. Splenic enlargement, therefore, is obviously the result of extraneous factors, and can often be traced to mouse typhoid, as has been proved in the first part of this paper. In a few instances, it is undoubtedly due to a condition resembling leukemia in the human subject.

The absence of any connection between immunity and splenic hypertrophy cannot, of course, be used to rebut the argument of such observers as Da Fano (34) and Murphy and his collaborators (35), who regard the lymphocyte as an important link in the process of immunization. For it is obvious that the spleen might be actively engaged in bringing about the refractory state, and yet show no evidence of its participation in so far as its size is concerned.

Although not quite germane to the subject discussed in the present paper, because they deal with spontaneous neoplasms,

the observations of Ewing (36) are nevertheless of interest in this connection. As the result of his own and others' experience at the autopsy table, this author denies any uniform relation between the size and condition of the spleen and the presence of cancer in the human body, and goes on to point out that cancer is, in general, a disease of elderly persons, in whom the lymphoid tissues and the spleen might reasonably be expected to show some physiological decline as compared with youthful subjects. On the other hand, actively growing tumors may be found in young patients with abundant lymphoid tissue. His conclusion that no information can be drawn from post mortem experience to favor the hypothesis that any part of immunity to spontaneous new growths is referable to the lymphatic apparatus, is shared by Wells (37), who has observed that spontaneous tumors frequently develop in mice with lymphatic or myelogenous leukemia; and that even where there is a prodigious increase in the number of lymphocytes, the growth is entirely unaffected.

These observations of Ewing and of Wells are not quite analogous to those discussed in the present paper because they deal with spontaneous new growths, and an animal cannot be immunized against his own tumor, as Haaland (38) has shown. An interesting experiment has been reported by Wood (39), who found that a transplanted tumor grew quite as well in a leukemic mouse as it did in a normal one. Still this case is not quite identical with those discussed in the experimental portion of this paper, because a mouse cannot be immunized with its own tissues (40, 41, 42).

This is, indeed, a fundamental weakness in Murphy's work—that animals so far have been immunized only with the tissues of others of the same species, never yet with their own. Therefore if the lymphatic system of a mouse is able to protect the animal against a new growth, it must be through the induction of some sort of immunity with which we have no acquaintance at the present time.

SUMMARY

While it is not denied that the spleen is concerned in bringing about immunity to propagable neoplasms, there is no evidence to show that the refractory state in mice is regularly accompanied by any enlargement of this organ appreciable to measurement. Some immune mice have enlarged spleens and some have not; some animals with progressively growing tumors have enlarged spleens and some have not. And the existence of other causes of splenic hypertrophy, such as mouse typhoid, transfers the burden of proof to those who assert that splenic hypertrophy is referable to immunity.

REFERENCES

- (1) CIMORONI: Tumori, 1911-12, i, 691.
- (2) BRANCATI: Tumori, 1911-12, i, 189.
- (3) PRICE-JONES: Arch. Middlesex Hosp., 1911, xiii, 56.
- (4) MAZZA: Tumori, 1914, iv, 257.
- (5) PRICE-JONES: Arch. Middlesex Hosp., 1913, xxx, 162.
- (6) APOLANT: Ztschr. f. Immunitätsforsch., 1913, Teil 1, Orig., xvii, 219.
- (7) v. HANSEMAN: Deutsch. med. Wchnschr., 1915, xli, 633.
- (8) KETTLE: Jour. Path. and Bacteriol., 1912-13, xvii, 40.
- (9) GRAF: Centralbl. f. Path., 1910, xxi, 723.
- (10) GOLDMANN: Beitr. z. klin. Chir., 1911, lxxii, 1.
- (11) ENDLER: Ztschr. f. Krebsforsch., 1915, xv, 339.
- (12) BRANCATI: Tumori, 1911-12, i, 189.
- (13) APOLANT: Ztschr. f. Immunitätsforsch., 1913, Teil 1, Orig., xvii, 219.
BRANCATI: Tumori, 1912-13, ii, 74. Cited by Apolant.
- (14) BULLOCK AND ROHDENBURG: Jour. Cancer Research, 1917, ii, 465.
- (15) OSER AND PRIBRAM: Ztschr. f. exper. Path. u. Therap., 1912-13, xii, 295.
- (16) BRANCATI: Tumori, 1912-13, ii, 74.
- (17) ROHDENBURG, BULLOCK, AND JOHNSTON: Arch. Int. Med., 1911, vii, 491.
- (18) BIACH AND WELTMANN: Wien. klin. Wchnschr., 1913, xxvi, 1115.
- (19) MORRIS: Jour. Cancer Research, 1917, ii, 498.
- (20) MOTTRAM AND RUSS: Proc. Roy. Soc., Series B, 1917, xc, 1.
- (21) BULLOCK AND ROHDENBURG: Jour. Cancer Research, 1917, ii, 465.
- (22) BRAUNSTEIN: Berl. klin. Wchnschr., 1911, xlviii, 2029; Ztschr. f. Immunitätsforsch., 1913, Teil 1, Orig., xviii, 330.
- LEWIN AND MEIDNER: Ztschr. f. Krebsforsch., 1912, xi, 364.
- (23) MORRIS: Jour. Cancer Research, 1917, ii, 498.
- (24) MOTTRAM AND RUSS: Proc. Roy. Soc., Series B, 1917, xc, 1.
- (25) BIACH AND WELTMANN: Wien. klin. Wchnschr., 1913, xxvi, 1115.
- (26) DA FANO: Ztschr. f. Immunitätsforsch., 1910, Teil 1, Orig., v, 1; Fifth Sci. Report, Imperial Cancer Research Fund, London, 1912, p. 57.

- (27) WOGLOM: *Ztschr. f. Immunitätsforsch.*, 1911, Teil 1, Orig., xi, 683.
- (28) *Memoirs of the Wistar Institute of Anatomy and Biology*; No. 6; The Rat. Compiled and edited by H. H. Donaldson, Philadelphia, 1915.
- (29) MEDIGREANU: *Proc. Roy. Soc.*, Series B, 1910, lxxxii, 286; *Berl. klin. Wchnschr.*, 1910, xlvii, 588.
- (30) BASHFORD: *Tr. XVII Internat. Cong. Med.*, London, 1913. Sub-Section III (a), *Chemical Pathology*, p. 29.
- (31) RONDONI: *Ztschr. f. Immunitätsforsch.*, 1913, Teil 2, Ref., viii, 959.
- (32) LOEFFLER: *Centralbl. f. Bakteriol.*, 1892, xi, 129.
- (33) RUSSELL: *Fifth Sci. Report, Imperial Cancer Research Fund, London*, 1912, p. 1.
- (34) DA FANO: *Ztschr. f. Immunitätsforsch.*, 1910, Teil 1, Orig., v, 1; *Fifth Sci. Report, Imperial Cancer Research Fund, London*, 1912, p. 57.
- (35) MURPHY AND STURM: *Jour. Exper. Med.*, 1919, xxix, 1 (bibl.).
- (36) EWING: *Jour. Cancer Research*, 1917, ii, 502; *ibid.*, 1919, iv, 59.
- (37) WELLS: *Jour. Cancer Research*, 1919, iv, 57.
- (38) HAALAND: *Fourth Sci. Report, Imperial Cancer Research Fund, London*, 1911, p. 1.
- (39) WOOD: *Jour. Cancer Research*, 1919, iv, 57.
- (40) WOGLOM: *Ztschr. f. Immunitätsforsch.*, 1911, Teil 1, Orig., xi, 683.
- (41) APOLANT AND MARKS: *ibid.*, 1911, x, 159.
- (42) LAMBERT: *Proc. Soc. Exper. Biol. and Med.*, 1911-12, ix, 18.

MULTIPLE TUMORS OF THE MOUSE MAMMA: ARE THEY INDEPENDENT OR METASTATIC?

ALBERT FISCHER

From Dr. F. G. Gades Pathological Institute, Bergen, Norway

Received for publication, May 15, 1919

Malignant neoplasms in human beings begin, as a rule, as single tumors, and the multiple growths which later appear are, as is well known, due to secondary spreading from this primary focus. Still multiple primary tumors are now and then to be met with; in the articles of Harbitz (1), Tanberg (2), de Besche (3), and Wolff (4), large series of such cases have been collected and discussed.

Harbitz arranges four groups of such multiple tumors:

1. Tumors of the same nature and in the same organic system.
2. Tumors of the same nature but in different organs.
3. Tumors of different nature and in different organs.
4. The carcinosarcoma.

Most of these tumors showing primary multiplicity are benign; the genuine malignant tumors, on the contrary, are less often primarily multiple in human beings.

To a certain degree the case appears to be otherwise with certain animal tumors, especially those in the mamma of mice, which are often apparently multiple. There are frequently found several independent growths in different parts of the mammary apparatus, which, in mice, is spread over a great deal of the body. Apolant (5) found in 221 cancerous mice about 12 per cent with multiple tumors; Murray (6), among 119 mice, about 15 per cent; and Haaland (7), in 288 mice, about 17 per cent. Multiple primary tumors appear to occur still more frequently in a strain of mice at Dr. F. G. Gades Pathological Institute (about 600 tumor mice), which will be described in a future publication from this laboratory. In almost all these cases the tumors were adeno-

carcinomata, appearing at widely separated points in the mammae, and thus belonging to Harbitz's group 1. In their multiplicity these tumors differ from those of human beings, and they have therefore been closely examined to see whether they actually are multiple primary tumors, or metastatic growths from a single primary focus.

When the various tumors show a completely different histological structure, there can be no doubt of their primary multiplicity. Often, however, they are similar and it is the latter case which will here be taken up, for the possibility that these are metastatic cannot be easily dismissed. To small histological differences much importance cannot be attached, as there are many cases where secondary tumors present considerable histological differences from the primary. Here it will be possible only in exceptional instances to produce proof that the growths are really multiple, and often one must be content with a probability. Where several rudimentary tumors appear at the same time and display about the same size, it is considered that one has a certain right to regard them as primary, and this is the case with the greater bulk of the cases which are reckoned as multiple tumors. Where there is a difference in the time of their appearance, or a considerable difference in their size, the matter will be much more difficult. But where the new tumors appear at widely separated points in the same organ system (mammary), and in the absence of metastases in other localities, especially in the interjacent tissues, it is more than probable that they are of wholly independent origin. For it is not likely that, if such tumors really are metastases, they should appear only in distant mammae, particularly as there does not appear to be any direct means of communication (Haaland (7)). If these cases can be included, the frequency of multiple tumors in mice will be considerably greater than is expressed by the figures previously mentioned; but one must exert his critical faculties to the utmost to exclude the possibility of metastasis.

Thus when all is considered, it appears probable that the multiple tumors of mice are independent, and not metastatic growths, though no direct proof has been produced. As the point is of

considerable interest, and our knowledge of the anatomical conditions upon which the question turns are defective, an attempt has been made to examine these conditions somewhat more closely. The method employed was the injection of insoluble particles into the mamma and a study of their transportation and deposition, as it may be assumed that tumor cells will in all likelihood follow the same path.

The technic was as follows: India ink was injected into the breasts through the nipple by means of a small syringe with a very fine needle. The quantity injected was about 0.1 cc. Female mice which had developed spontaneous tumors in one or more mammae were used for the experiments. In some of the animals the injection was made into one of the still normal breasts, in others into the tumor itself. The ink was introduced by preference into the inguinal mammae, which were found easier to inject.

The results were as follows (cf. table):—

1. When India ink was injected into the normal breast, it always appeared in the regional lymph-nodes. At first the periphery, later the entire node, was of a more or less intense black color.

2. In the next stage the course of the India ink could be followed to the central nodes and on to other lymph-nodes on the same side, and in individual cases ink has been observed in nodes on the opposite side.

3. No ink has ever been found in another mamma.

4. When India ink was injected into mammary tumors, the same occurrence was noted, except that transportation took place much more rapidly, both to the regional nodes and to those lying above and below them. Here, too, ink was never transported to other mammae.

The conclusion may be drawn that small solid particles, like those of India ink, travel from the mamma through the lymph-channels to the regional nodes, and then on to more distant lymph-nodes, as they do in the human subject. Direct transport from one breast to another, such as might permit multiple tumors to be regarded as of metastatic origin, has not been observed.

TABLE 1

NUM- BER	SITE OF INJECTION		KILLED AFTER IN- JECTION	RESULTS			
	Mamma	Tumor		Regional nodes	Other nodes on same side	Nodes on opposite side	Other organs
			<i>days</i>				
512		R. ing.	1	+	Mediastinal and ab- dominal.	—	—
521	L. axil.	—	1	+	—	—	—
517	L. ing.		1	+	Abdominal.	—	—
524	L. ing.		2	+	Axil., abdominal.	—	—
527	L. ing.		2	+	Abdominal, axil.	Ing.	—
534		Back	2	—	—	—	—
461		L. vul- var.	2	+	Axil.	—	—
460	R. ing.		2	—	—	—	—
475	R. ing.		2	+	Axil., abdominal.	Axil.	—
485	L. ing.		3	+	Axil.	—	—
496	R. ing.		3	+	Abdominal, medi- astinal.	—	—
542	R. axil.		3	+	Axil., cerv.	Axil.	—
531	L. ing.		3	—	Axil.	—	—
533	R. ing.		3	+	Mediastinal, axil.	Ing., axil.	—
526	R. axil.		4	+	—	—	—
525	L. axil.		4	+	—	—	—
536*	R. ing.		4	+	Axil.	—	—
532	L. ing.		5	+	Abdominal.	—	—
535		L. ing.	5	+	Mediastinal, axil.	—	—
540	L. ing.		5	+	Abdominal, axil.	—	—
543	R. ing.	L. ing.	5	++	—	—	—
490	L. ing.		5	+	—	Axil.	—
497	R. vul- var.		5	+	Mediastinal, axil.	—	—
483		L. ing.	11	+	Axil.	—	—
491	L. ing.		11	+	—	—	—
480		L. ing.	15	+	—	—	—
501	R. ing.		15	+	Abdominal, axil.	Axil.	—

* In this case the India ink wandered directly from the inguinal lymph-nodes through a lymph-channel in the skin up to the axillary nodes, without the central (abdominal) nodes being blackened. Here there was no black coloration of the interjacent breast-tissues.

Therefore the multiple new growths which appear so frequently in the mammae of mice are to be explained as independent, rather than as metastatic tumors.

- (1) HARBITZ: Norsk Mag. f. Lægevidenskaben, 1916, p. 77.
- (2) TANBERG: *ibid.*, 1906, p. 983.
- (3) DE BESCHE: *ibid.*, 1908, p. 540.
- (4) WOLFF: Die Lehre von der Krebskrankheiten, Jena, 1911, II Teil, 406.
- (5) APOLANT: Cit. by Haaland.
- (6) MURRAY: Cit. by Haaland.
- (7) HAALAND: Fourth Sci. Report, Imperial Cancer Research Fund, London, 1911, p. 23.

ATTEMPTS TO OBTAIN A TRANSPLANTABLE TUMOR IN THE HIGHER SPECIES OF ANIMALS

F. C. MANN

Department of Experimental Surgery, Mayo Clinic, Rochester, Minnesota

Received for publication, March 24, 1919

Many attempts have been made by different investigators to transplant tumors in the higher species of animals, but all such experiments, with the exception of those dealing with the infectious sarcoma of the dog, have been failures. As the number of animals employed in any particular series has always been small, the chances of obtaining a successful growth have not been so good as if a larger series had been made. The advantage of obtaining a transplantable growth in such a species as the dog is quite obvious.

The purpose in the present article is to report a series of experiments in which attempts were made to transplant tumors of the dog and cat. It was thought that the chances of success would be greatly enhanced if a large series of animals was employed. The data obtained seemed to be of sufficient value to justify publication.

1. METHOD OF EXPERIMENTATION

After a preliminary investigation of the best method of transplanting tissue from one animal to another, a technic was devised as follows: Under anesthesia, usually local, and with the employment of careful sterile technic, the specimen was removed from the tumor and immediately cut with a very sharp razor into slices about 1 mm. thick. These slices were spread on a glass or a cork surface. A trocar having an internal diameter of about 2 mm. was loaded with a piece of tumor by pressing its sharpened distal end into the tissue against the underlying surface of

glass or cork. Each transplant was therefore as thick as the original slice and of the same diameter as the internal diameter of the trocar. The stylette of this trocar had a blunt end. The skin of the area chosen for transplantation was prepared in the usual manner by shaving and treating with iodine, and a second trocar with a sharp stylette was then plunged into the tissue to the desired point; this second trocar had an internal diameter of a size that would just freely admit the passage of the first. After withdrawal of the sharp stylette, the small trocar was plunged into the same position, and the stylette of this trocar was used to push the transplant well out into the tissues. After withdrawing both trocars the wound was treated with iodine. The transplant thus injected was disk-shaped and measured approximately 2 x 1 mm. In the employment of this technic it is possible, with the aid of three assistants, to make thirty to forty transplantations in the course of an hour, and rigidly to observe the rules of sterile technic; moreover, a better chance of obtaining a viable transplant will be insured.

Since we desired to make the investigation as extensive as possible, the site of transplantation varied. Usually one graft only was injected into an animal, but in some instances two were employed, often from different portions of the tumor.

II. THE RESULTS OF TRANSPLANTING A MAMMARY CARCINOMA IN THE DOG

The donor in this series of experiments, a well bred Scotch collie, was given to Dr. C. H. Mayo for the purpose of having this investigation made.

Protocol of the donor, dog C185. The animal was admitted to the laboratory on October 25, 1917. Though old, the animal was in excellent condition and weighed 18.6 kgm. Examination showed a large, hard, nodular tumor measuring 8 cm. in diameter, protruding about 2 cm. outward on the right side of the abdomen, and involving the third mammary gland on that side. Pus could be expressed from a central aperture, about 1 cm. in diameter, the end of a sinus which seemed to extend to the base of the tumor. The adjacent axillary lymph-

node on the same side as the tumor was enlarged and hard. On October 31 a specimen of the tumor was removed and examined microscopically; it proved to be carcinoma. On November 1, portions of the tumor were removed and injected into 54 other dogs. Although in the first 15 animals the trocars used were rather large, for all subsequent transplantations the method here described was employed. On November 8 the enlarged lymph-node was removed; it was found greatly enlarged, measuring approximately 3 x 1 cm., and was very firm, while at the lower pole there was a white nodule about 8 mm. in diameter which

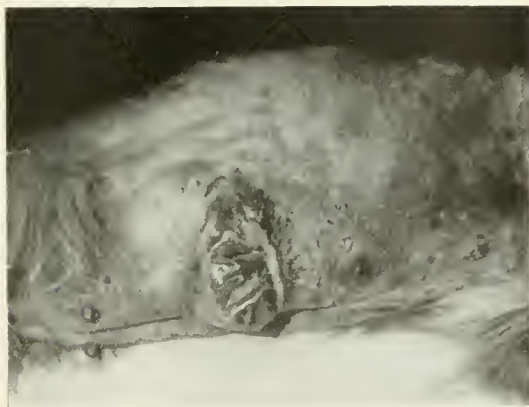


FIG. 1. PHOTOGRAPH OF DOG C 185 SHOWING TUMOR OF MAMMARY GLAND FROM WHICH THE TRANSPLANTS WERE MADE

looked like a metastasis. Transplants of this nodule were injected into 19 dogs, and one injection was made into the first mammary gland in the right side of the donor. On November 9 portions were again removed from the primary growth and transplanted into 24 other dogs, after which an injection was made into the first mammary gland on the left side of the donor. On November 24 fragments from the primary growth were transplanted for a third time, into 34 other dogs, and into the second and fourth right and third and fourth left mammary glands of the donor.

The animal remained in excellent health until November 25, when she became quite sick, refused food, and lost weight and strength. The scleras and mucous membranes became distinctly tinged with yellow. On December 2 the animal was bled to death under ether anesthesia, and necropsy (636-17) was performed immediately. The original growth was comparatively small. The only transplants to be found were in the fourth breast on the right side, and the third and fourth on the left side; at each of these three sites, areas were found in which there appeared to be viable transplants. The superficial and

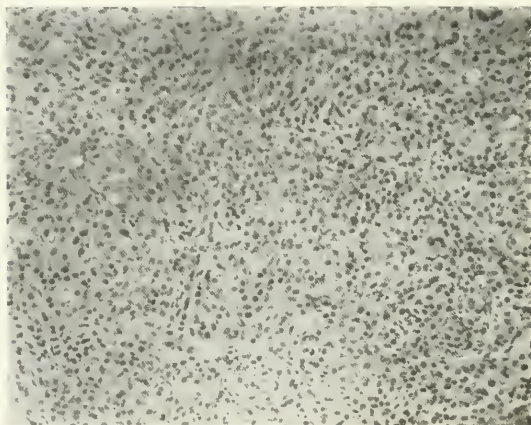


FIG. 2. PHOTOMICROGRAPH OF ORIGINAL TUMOR IN DOG C 185 ($\times 125$)

deep lymph-nodes on the left side were slightly enlarged, but did not appear to contain carcinoma. The thyroid had undergone cystic degeneration, and very little normal thyroid tissue remained. No thymic tissue could be found. The heart was flabby but otherwise appeared normal. The lungs exhibited many metastases ranging from 2.5 cm. to 2 mm. in diameter, every lobe containing one or more. In the lower part of the anterior mediastinum was a large irregular mass, which measured approximately from 10 to 12 cm. in diameter and was composed wholly of carcinoma. The duodenum and pancreas were adher-

ent to the peritoneal wall opposite the site of the original tumor. The gastro-intestinal tract was practically empty and normal, except for a few small superficial ulcers. The pancreas was studded throughout with small areas which appeared somewhat like fat necrosis but which, owing to their similarities and discreteness, were considered to be metastases; their size varied from 1 to 4 mm. in diameter. The liver was also studded with nodules, apparently metastatic, varying in size from 1 to 2 mm. to 2 cm. in diameter. The spleen and the right kidney appeared normal, but the left kidney contained a large metastatic

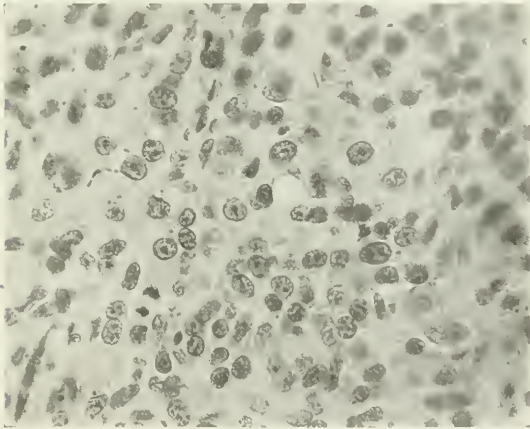


FIG. 3. PHOTOMICROGRAPH OF ORIGINAL TUMOR IN DOG C 185 ($\times 500$)

nodule in the pelvis, measuring about 1 cm. in diameter, and two small ones in the cortex each about 3 mm. in diameter. The left adrenal also showed a small metastasis. No other pathologic findings were noted.

The specimens were fixed in formalin and neutral formalin and Zenker. Histologically the primary growth was a typical mammary carcinoma. Metastatic carcinoma cells were found in the axillary lymph-node from which transplants were made, in the lungs, mediastinal lymph-nodes, liver, pancreas, kidney, and adrenal. The three transplants which at autopsy appeared to be viable did contain viable carcinoma cells.

Conditions and results of the injection experiments

Since it was our purpose to attempt to produce a transplantable tumor, as many animals as possible were injected. No attempt was made to study the immediate fate of the transplant, and end results only were observed.

1. *Number of injections.* The total number of dogs injected was 134; five of the animals were inoculated twice, so that the total number was 139, not including those made into the donor. One hundred and twenty were of the primary growth and 19 of the axillary lymph-node metastasis.

2. *Infections.* Owing to the fact that the primary growth was infected at the time the animal entered the laboratory, great difficulty was encountered in getting sterile specimens for transplantation. Infection, however, did not prove to be a very serious factor; there were ten definite infections, some of them severe, at the site of implantation, and eighteen slight infections. That these latter were not of great importance is proved by the fact that viable carcinoma cells were noted in some of the cases classified as "slightly infected." In 111 cases the healing was primary.

3. *Site of injection.* One hundred and eight of the injections were made subcutaneously in the left axillary region, which was chosen most often because it is easy to inject and to observe. The mammary gland seemed to be another favorable site, and 15 injections were made there, though of course in some instances the transplant did not penetrate into the substance of the gland. In puppies it was easier to inoculate into the groin, and 16 injections were made in that area. Three injections were made in the middle of the left nipple line, and one each into the testicle, into the peritoneal cavity, and into a granulating wound.

4. *Age of the animals injected.* The larger number of the dogs were young adults, although practically all ages were represented; the youngest were puppies only a few weeks old; a few were very old, particularly one, in whose mouth only six teeth remained.

5. *Condition of the animals injected.* Most of the dogs were in excellent condition, though a few were in very poor health, either because they had but recently been received in the laboratory, or because they were suffering from infection. Two or three of the animals had distemper at the time of injection.

6. *Breed of the animals injected.* A majority of the animals were mongrels with a preponderance of the characteristics of one particular breed. Several of them, however, were well bred. As the donor was a collie, it seemed desirable to employ as many collies as possible; four of the twelve such animals injected were pedigreed. Several of the animals of other breeds were known to be of pure stock.

7. *Operative procedures to which the animals were subjected before injection.* Approximately half of the animals employed in the series had been previously subjected to operative procedures. In most instances the operations could not have had any relation to a positive or negative result in the transplantation of the tumor, though in some cases the operation was regarded as a possible complicating factor. For example, in a few animals one ovary had been removed, one animal had been castrated before he was received at the laboratory, one had received many exposures to the x-ray, five had been splenectomized before, and four after inoculation.

8. *The agglutinins in the recipients' blood.* It was thought that possibly the different animals could be grouped like human beings with regard to agglutination of the blood, and that this grouping might have something to do with the growth of the transplant. Accordingly the corpuscles of 65 dogs were tested with the serum of the donor. Agglutination occurred in two instances only, which probably indicates that dogs cannot be grouped.

9. *Results of transplantation.* After injection the animals were closely observed. Some of them died from various causes, and others were used in subsequent experiments; thus most of them came to necropsy although several are alive one year after injection. In only six animals was the transplant found and in two of these it was definitely dead and necrotic. In four instances visible cancer cells were found at necropsy.

Protocols of experiments in which viable carcinoma cells were found at necropsy

1. Dog C29, a small, black, male terrier, weighing 7.6 kgm. was admitted to the laboratory in June, 1917. On June 22 Dr. Harrington sectioned and anastomosed the right ureter (experiment 453-17). The animal did very well immediately after the operation, but developed later a superficial abscess at the site of some non-absorbable suture material, gradually lost weight; in October the abscess was

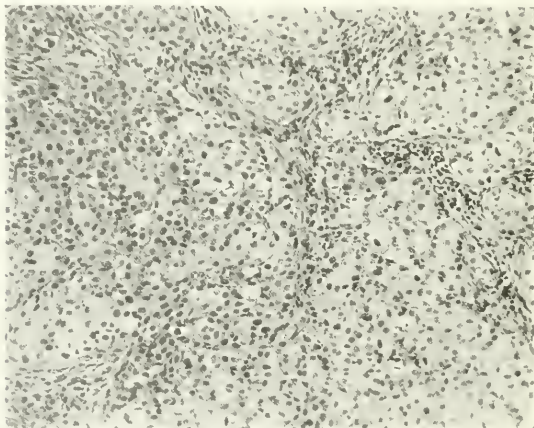


FIG. 4. PHOTOMICROGRAPH OF TRANSPLANT OF DOG C 28

Compare with original figure 2 ($\times 125$)

opened and the foreign material removed. On November 1 two pieces of carcinoma, measuring approximately 2 by 2 by 1 mm. were removed from the donor (dog C185) and injected into the left axilla within less than one hour. The trocar wound closed, but on November 4 it seemed to be infected and a slight amount of seropurulent fluid escaped; there was, however, very little reaction around the site of injection. On November 9 the wound was healed, and the transplant could not be found. A small palpable nodule developed at the site of the transplant, but it could not be differentiated from a

lymph-node. The animal remained in poor condition and died suddenly on December 11, forty-one days after the injection of the carcinoma specimen.

This experiment came the nearest to success. At necropsy (612-17), which was performed shortly after death, a nodule could be palpated at the site of the injection. On dissection this was found to be a small discrete mass, light in color and appearing very much like the primary carcinoma of the donor. It was roughly elliptical in shape, measured

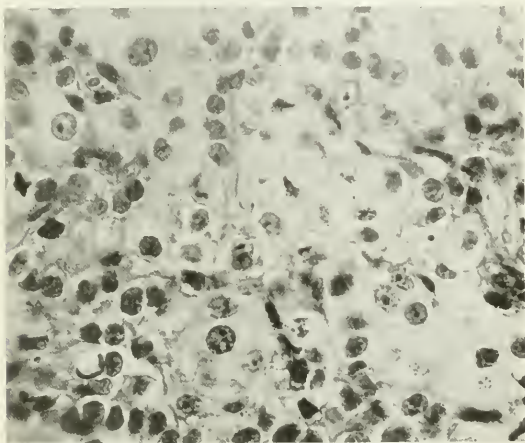


FIG. 5. PHOTOMICROGRAPH OF TRANSPLANT OF DOG C 28

Compare with original figure 3

4.3 by 3 by 3 mm., and had a capsule of its own and a well developed blood supply. It was very easily exposed and freed from surrounding tissue, and appeared to be the transplant.

The tissue was fixed in formalin and paraffin serial sections were made. Microscopically the tissue was shown to be the transplant, completely surrounded by a definite capsule. The carcinoma cells in many areas were situated in small groups, which were surrounded by well developed connective tissue, though there were small areas where the carcinoma cells were packed close together with very little intervening

connective tissue. In many areas there was distinct round cell infiltration, often following the bands of the connective tissue and thus penetrating between the groups of carcinoma cells. The carcinoma cells, especially in the areas which were infiltrated with round cells, were vacuolated and fragmentary. There were many large areas, particularly those in which the cells were packed closely together and in which the carcinoma cells were perfectly normal in appearance. In these areas it was possible to find a few carcinoma cells undergoing mitotic cell division. In this experiment there is no question but that the transplant lived and grew. In the forty-one days it remained in the new host it had more than quadrupled its volume, although it was walled off by a definite capsule. There was well marked evidence of beginning absorption, shown by the round cell infiltration and the vacuolization of the cells. On the other hand, many of the carcinoma cells were in excellent condition and a few mitotic figures showed that growth was still taking place. Whether the transplant would have grown much larger if the animal had lived longer is questionable; in all probability it would gradually have been absorbed. The other three experiments in which a viable transplant was found at necropsy will be briefly described:

2. Dog C240 died twelve days after the injection. The transplant was found to be undergoing necrosis and absorption; only a few viable carcinoma cells were present.

3. Dog C252 died twelve days after the injection. The transplant was undergoing necrosis and absorption, and only a few viable carcinoma cells were present.

4. Dog B981 died seven days after the injection. Most of the cells of the transplant were becoming necrotic although there were undoubtedly many viable carcinoma cells near the edge of the transplant.

It may be noted that in each of these three experiments the length of time between the injection of the transplant and the death of the animal was short.

III. THE RESULTS OF TRANSPLANTING A FIBROMA IN THE CAT

Protocol of the donor, cat 113. This animal, a yellow and white male weighing 2452 grams was admitted to the laboratory on December 22, 1917, in rather poor condition. In August, 1917, the owner had noticed

a small nodule on the external surface of the left ankle. This nodule grew and soon ulcerated, and at the time of examination measured about 2 by 2 cm., the whole external surface was ulcerated, the growth



FIG. 6. PHOTOGRAPH OF CAT 113 SHOWING TUMOR ON LEG
Note the small nodule above tumor; this is the first transplant



FIG. 7. PHOTOGRAPH OF VIABLE AXILLARY TRANSPLANT IN CAT 113
The two white masses embedded in muscle are the transplants (natural size)

appeared to be firmly attached to the deeper structures and the infection extended deep into its substance. A small piece was removed for histologic study. On December 26 several pieces of the tumor were

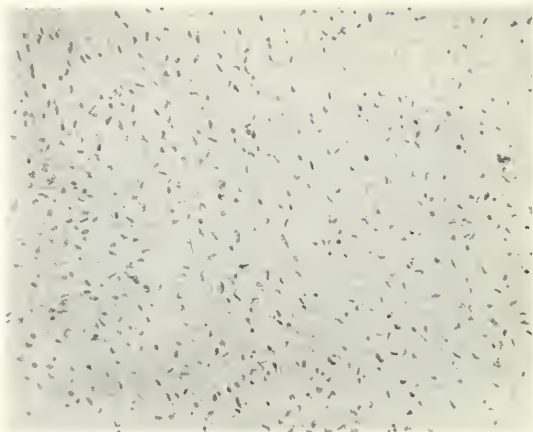


FIG. 8. PHOTOMICROGRAPH OF ORIGINAL TUMOR IN CAT 113 ($\times 125$)

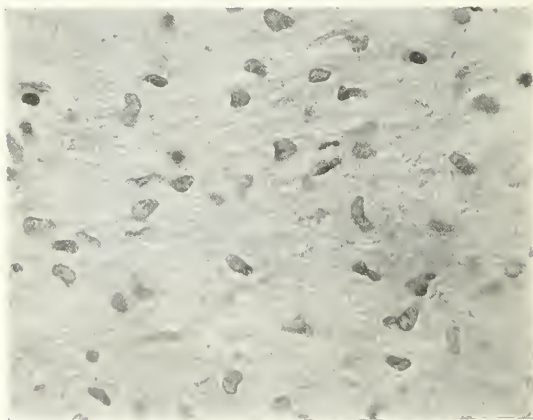


FIG. 9. PHOTOMICROGRAPH OF ORIGINAL TUMOR IN CAT 113 ($\times 500$)

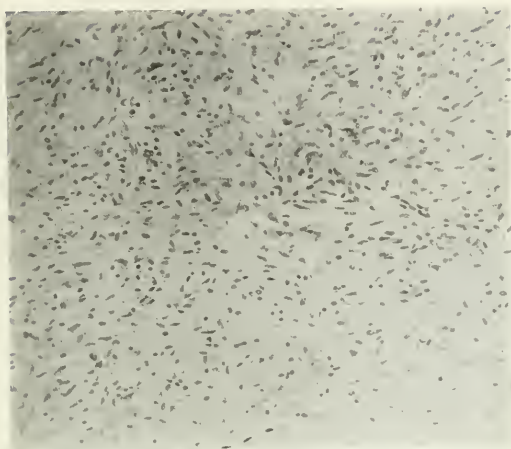


FIG. 10. PHOTOMICROGRAPH OF AUTOTRANSPLANT IN LEG OF CAT 113 ($\times 125$)

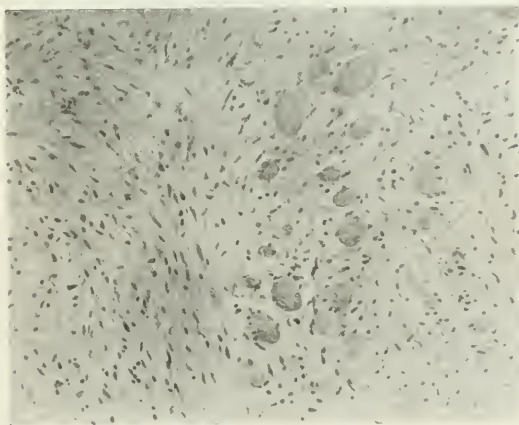


FIG. 11. PHOTOMICROGRAPH OF AUTOTRANSPLANT IN AXILLA OF CAT 113
Note bundle of muscle in the growing transplant

removed and transplanted into eight other cats, though it was practically impossible to obtain sterile pieces of the tumor. One transplant was made subcutaneously in the donor just above the original growth. Subsequently the ulcerated surface of the tumor was treated and all but the superficial infection was cleared up. The tumor continued to grow, and on January 29, 1918, it measured 4.5 by 3 by 3 cm. At this time sterile pieces could be obtained, and transplantations were made into seven cats, after which two transplants (two pieces) were injected



FIG. 12. PHOTOMICROGRAPH OF TRANSPLANT IN CAT 143, 76 DAYS AFTER TRANSPLANTATION

(Compare with original figure 8 ($\times 125$))

into the donor (cat 113), one into each axilla. The trocar wound on the left side, which became infected, necessitated drainage.

The animal remained in good condition, the tumor grew, and the ulceration under treatment remained localized. On February 11, specimens were transplanted into five cats. The transplants injected into the axillae of the donor could not be palpated, and after the five cats had been injected, two more transplantations were accordingly made into the right axilla of the donor. The transplant that had been

injected into the leg of the donor on December 26 could be palpated. The primary tumor grew slowly and the ulcerated surface healed, but the general condition was not good; the animal lost strength and weight and became anemic. On June 10 fragments were removed and injected into four rats. The donor was then etherized and bled to death.

Neeropsy (289-18) was performed immediately after death. The original tumor extended rather deeply, but was not connected with the bone; it was encapsulated, though it could be dissected free

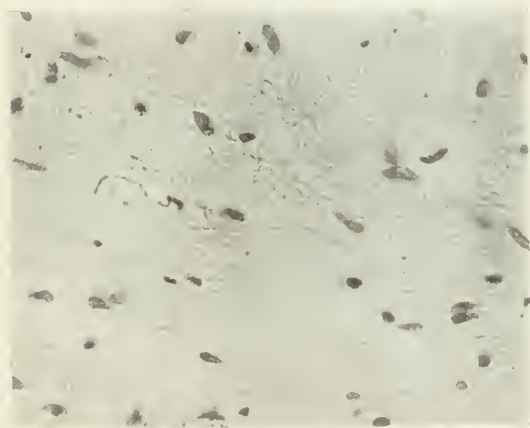


FIG. 13. PHOTOMICROGRAPH SHOWING A HIGHER POWER OF THE SAME SECTION AS FIGURE 12

Compare with figure 9 ($\times 500$)

from the surrounding tissue only with difficulty. No metastases could be found: the lymph-nodes near the tumor were enlarged, but this was due to the infection. All the organs appeared normal. The first transplant, injected subcutaneously above the original tumor on the same leg, had grown, and measured 8 mm. in diameter; it was discrete and appeared similar to the primary growth. Two small nodules could be palpated in the left axilla. These when exposed were found buried in muscle and had the same appearance as the mother tumor;

they measured 7 by 6 by 6 mm. and 6 by 5 by 5 mm., respectively. They were undoubtedly the transplants that had been injected January 29. The transplants in the right axilla were found, but they were quite small, although they appeared viable.

The specimen that was removed from the tumor on December 22 was taken at a point close to the infected surface. Histologically it appeared to be a sarcoma. However, examination of specimens taken at a point somewhat removed from the ulcerated surface, and of all specimens removed after treatment, showed the growth to be a fibroma. The transplants appeared similar, histologically, to the mother tumor.

Conditions and results of the experiments

1. *Number of injections.* The total number of cats injected with transplants from the original tumor was 25. One animal was injected at two different times. The number of animals injected with a transplant from previous transplants was 7; total number 32. Many of the animals had more than one transplant injected; the total number of inoculations was 46.

2. *Infection.* When the donor was received at the laboratory the tumor was infected, which made it practically impossible to obtain sterile specimens. For that reason the first series of transplants had a very high incidence of infection. After the ulcerated surface had been treated and sterile specimens could be obtained, infection did not occur. Of a total of 46 injections only 6 were infected. It should again be noted that infection does not greatly decrease the chances of a viable transplant, for one of the trocar wounds in the donor (cat 113) became so badly infected that it required free drainage, yet the transplant grew.

3. *Site of injection.* The majority of the inoculations were made subcutaneously in the axillary region, over the xyphoid process, or near the umbilicus. A large number, however, were made into the peritoneal cavity.

4. *Age.* The youngest animal injected was about two thirds grown. A few of the animals were definitely aged, but most of them were young adults.

5. *Condition.* With one or two exceptions, the general condition of all the cats was excellent. None of the animals had been subjected to any previous operative procedure.

6. *Breed.* All the animals were mongrels.

7. *Results.* The total number of transplantations from the original tumor was 31. The transplant was found to have grown or remained viable in 12 cases. Two of these that appeared to be viable had been transferred for too short a time to be of any value in drawing conclusions. The other ten were found viable 9, 13, 18, 23, 25, 38, 46, 50, and 76 days after injection. All of these without doubt had grown in the new host. However, all that had remained long enough in the recipient had been gradually absorbed and there is no doubt that all would ultimately have been.

Transplants taken from a previous transplant were injected in 15 instances. Microscopically these transplants were found to contain at least a few viable tumor cells at the time of the second injection although in almost every instance death and absorption of the transplant were in progress. None of these sub-transplants remained viable.

8. *Results of reimplantation of the tumor in the donor.* After each series of transplants had been injected, one or two injections were made in the donor; thus five transplants were injected at three different times. Four of these grew. Only two, however, developed into tumors which were exactly similar in appearance, both grossly and histologically, to the mother tumor.

SUMMARY

The results are given of a series of experiments in which transplants of a mammary carcinoma of a dog were injected into 134 dogs, and of a series of experiments in which transplants of a fibroma of a cat were injected into 32 cats. A transplantable tumor was not obtained in either series of experiments, although a few transplants grew for a short time. The transplants of the fibroma which were made in the donor grew. The results obtained in these experiments, in which tumor was employed, are strikingly similar to the results of auto- and homo-transplantation of normal tissue. The problem of developing a transplantable tumor in the higher species of animals is, it would seem, closely allied to the problem of making homo-transplants of normal tissue grow.

A STUDY OF THE CHEMICAL COMPOSITION OF THE BLOOD IN CANCER

RUTH C. THEIS AND WILLIAM S. STONE

*From the Huntington Fund for Cancer Research, Memorial Hospital, and the Harri-
man Research Laboratory, Roosevelt Hospital, New York*

Received for publication, June 4, 1919

Since the micro-methods of chemical analysis have been developed, much work has been done on the chemical analysis of the blood in various pathological conditions, particularly in nephritis. Almost no work, however, has been done on the chemical analysis of the blood of patients suffering from cancer, although definite changes might be expected in a disease so marked by anemia and cell proliferation.

The sugar content of the blood in cancer has been a subject of discussion for many years. In 1885, Freund (1) regularly noted an increase, the figure being sometimes up to 0.33 per cent, which was confirmed by Trinkler (2). Matrai (3) maintained that such an increase was not characteristic of cancer but of the anemia which is secondary to the disease. Lewis and Benedict (4) using their new method, reported that 19 out of 53 cases of malignant disease showed a blood sugar figure above 0.11 per cent.

The alkalinity of the serum in malignant disease and other pathological conditions has recently been investigated by Maud Menten (5). In a series of 73 cases of malignant disease an increased alkalinity was noted in all except three or four. It was more pronounced in cancer of the internal organs than in superficial lesions. During pregnancy in which physiological cell-proliferation takes place, she found normal alkalinity. The increased alkalinity in cancer, however, is not specific for the disease, for it is found to be abnormally high in various skin diseases, and in diabetes mellitus in the stage preceding acidosis.

That the cholesterol content of the blood of cancer patients

is increased was reported by G. Luden (6), using the methods of Autenrieth and Funk (7) and Bloor (8). Considering the upper limit of cholesterol in normal blood to be 0.27 per cent, it was found in nine cases of cancer to vary from 0.26 per cent to 0.71 per cent, the lower values being found in cases that had been treated with radium. Denis (9), however, using the modified method of Bloor, reported a normal content in all but fourteen cases of early cancer, with the exception of one, in which a low content was associated with anemia.

We have determined sugar and the non-protein nitrogen constituents except creatinine and creatine in blood obtained from 189 patients in the wards of the Memorial Hospital, all of whom were suffering from malignant or allied disease such as leukemia or Hodgkin's disease. Many of the severe types of cancer were represented, and the majority of the cases were well advanced, so that changes in the blood should have been found if such changes are characteristic of the disease. The writers are indebted to the house staff of the hospital for their prompt and regular withdrawal of the blood. This was obtained from a vein in the arm each morning between 11.00 and 12.00, and defibrinated. As many of the constituents of the blood were determined on each specimen as the amount of blood withdrawn allowed. Duplicates were always done on non-protein nitrogen, urea, and sugar, and whenever possible on uric acid and amino acid nitrogen.

The methods used were:

Non-protein nitrogen: Greenwald's (10) modification of the Folin-Denis method. Distillation, according to Bock and Benedict (11) instead of aëration and direct titration with 0.01 N NaOH.

Urea: Marshall (12).

Uric acid: Benedict's (13) modification of the Folin-Denis method using the modified uric acid reagent (14).

Amino acid nitrogen: Bock (15).

Sugar: Lewis and Benedict (16).

For comparison of the figures obtained in the present study, it is necessary to consider briefly the normal value for the non-protein constituents of the blood.

As the result of the work carried out at Cornell Medical College and in certain other laboratories where the same methods have been used which we employed, we adopted the following figures as representative of normal values. Non-protein nitrogen, 28 to 35 mgm. per 100 cc. Urea nitrogen, almost exactly 50 per cent of the non-protein nitrogen, viz., 14 to 18 mgm. per 100 cc. Uric acid ranges between 1 and 2.5 mgm. per 100 cc.; amino acid nitrogen is about 7 to 8 mgm. per 100 cc., and sugar 90 to 110 mgm. per 100 cc.

In table 1 are presented the results of analyses of the blood from 189 cancer patients. The cases studied include a very wide range as regards type and malignancy of the disease. It is obvious that for any proper interpretation of the data presented account must be taken of the age of the patient, as well as of his general condition and the tissue involved in the disease process. Thus in cancer of the liver it would be quite unjustifiable to credit changes which might be found in the blood to the cancer process as such—these changes might be wholly due to disturbed metabolism resulting from improper functioning of the liver. Such objections would not apply to growths located in the breast or uterus, or to the various cases of purely superficial growths. Alteration of the blood constituents in such cases can properly be attributed to the cancer process, providing that these changes are apparent in a good percentage of the cases. The clinical data presented in table 1 have been condensed as much as possible, but it is hoped that enough of such data is included to enable one to judge whether the blood changes found can be ascribed to the disease, or must be referred to the impairment of some particular organ. The cases are classified in general groups according to the location of the disease.

An inspection of table 1 shows that the values found for non-protein nitrogen and urea are definitely below the normal. This is especially marked in the mammary and uterine cases, many of which show figures which are probably the lowest so far recorded for non-protein nitrogen and urea in human blood. Carcinomas of the breast and uterus are commonly recognized as representing the most malignant and rapidly growing tumors,

TABLE 1

NUMBER	AGE	NON-PROTEIN NITROGEN	UREA NITROGEN	URIC ACID	AMINO ACID NITROGEN	SUGAR	REMARKS
<i>Uterus</i>							
		mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	per cent	
1	40	28.0				0.088	Advanced early recurrence
2	31	26.6				0.073	Rapid. Terminal stage
3	55	30.8	14.7			0.110	Adenocarcinoma ovary. Recur- rent; long duration
4	45	30.8	14.7			0.112	Far advanced acanthoma. Pye- litis
5	52	32.2	12.6			0.104	Localized. Percy. Radium
6	35	28.0	13.6			0.088	Slowly growing recurrence
7	44	28.7				0.088	Well advanced. Rapidly grow- ing
8	49	25.2	14.0	1.2		0.111	Advanced. Short duration. Very malignant
9	53	32.9	15.4	3.3		0.100	Ovary. Advanced, but local
10	50	37.9	22.0			0.111	Superficial. Vulva
11	63	30.1	14.0	2.6		0.121	Long duration. Advanced
12	53	32.2	12.6	2.4	6.7	0.119	Advanced, but local
13	46	26.6	13.6	4.4	7.6	0.103	Short duration. Local. Im- munity
14	44	32.2	15.7	1.2	9.3	0.101	Duration 1 year. Advanced, but not very malignant
15	65	31.5	13.3	2.2	7.7	0.107	Local and limited adenoma
16	44	33.6	12.6		7.1	0.100	Early lesion, plus syphilis. Cured
17	37	25.2	10.1	1.9	7.2	0.102	Well advanced
18	44	30.8	12.6	4.3	7.5	0.117	Immunity. Recurrent. Exten- sive. Slow growing
19	42	28.0	16.1	3.3		0.089	Well advanced. Adenocarci- noma ovary
20	40	35.1	17.5		7.6	0.090	
21	45	28.0	10.5	5.3	7.1	0.145	Far advanced
22	48	30.8	12.9	4.0		0.153	Well advanced. Nephritis
23	47	21.6	9.1	2.9	7.1	0.107	Advanced
24	58	24.5	12.9		6.5	0.097	Far advanced. Long duration. General carcinosis
25	53	36.5	18.2		6.9	0.101	Rapid. Very malignant. Ad- vanced

TABLE 1—Continued

NUMBER	AGE	NON-PROTEIN NITROGEN	UREA NITROGEN	URIC ACID	AMINO ACID NITROGEN	SUGAR	REMARKS
--------	-----	-------------------------	------------------	-----------	------------------------	-------	---------

<i>Uterus.—Continued</i>							
		mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	per cent	
26	55	37.9	23.5		9.8	0.135	Very malignant. Extensive re- currence
27	35	22.4	10.1		8.3	0.089	Very malignant
28	34	25.9	17.5			0.091	Recurrence. Advanced. Im- munity
29	50	26.6	13.3		7.7	0.128	Very malignant. Kidneys
30	67	27.3	13.3			0.136	Local. Vulva. Degenerative changes of age
31	29	23.8	9.1		7.6	0.166	Advanced. Duration 1½ years. Hemorrhage
32	26	23.8	10.1		6.7	0.121	Very malignant. Rapid. Post- operative
33	32	25.2	10.8		7.6	0.106	Rapid. Very malignant
34	39	18.2		3.6			Short duration. Very malig- nant. Metastases
35	48	32.9		2.6			Metastases 5 weeks after opera- tion
36	38	16.8		<1			Early. Local; cured
37	53	30.8	18.2	1.4	10.5		Bad general condition. Recur- rence 3 years after operation
38	56	25.2	18.2		6.5		Advanced. Terminal stage
39	57	22.4		<1			Well advanced adenoacanthoma
40	48	22.4	5.6			0.107	Well advanced

<i>Breast</i>							
41	50	22.4				0.122	Rapidly growing. Late stage. Male
42	43	30.8	16.1	2.2		0.113	Recurrent; advanced
43	34	28.7	12.9	3.8	13.4	0.091	Anaplastic; rapid; pregnant. Very responsive to x-ray
44	27	24.5	10.5		8.1	0.105	Anaplastic; pregnant. Rapid. Responsive to radium for short period
45	38	23.8	12.6		7.3	0.100	Recurrent. Advanced
46	68	34.3	14.7	2.5	8.7	0.099	Early carcinoma. Nephritis
47	63	31.5	18.2	3.7	9.4	0.327	Slow growing. Nephritis. Dia- betic

TABLE I—Continued

NUMBER	AGE	NGN-PROTEIN NITROGEN	UREA NITROGEN	URIC ACID	AMINO ACID NITROGEN	SUGAR	REMARKS
--------	-----	-------------------------	------------------	--------------	------------------------	-------	---------

<i>Breast—Continued</i>							
		mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	per cent	
48	36	28.0	11.2		7.3	0.104	Rapidly growing. Advanced
49	48	26.6	13.3		6.5	0.104	Recurrent. Metastases. Advanced
50	52	28.0	11.2		9.8	0.135	Advanced. Immunity
51	46	25.9	11.9		6.1	0.094	Short duration. Metastases. Rapid advances
52	43	22.4	9.1		7.4	0.135	Very malignant. Metastases in femur. Nephritis
53	45	29.4	20.3	2.9			Far advanced. Fair general condition

<i>Mouth</i>							
54	45	25.2	13.3			0.092	Superior maxilla. Long duration. Advanced
55	41	25.2				0.137	Inferior maxilla. Short duration. Malignant
56	40	33.6	15.4			0.091	Carcinoma antrum
57	60	28.0	12.6			0.115	Carcinoma inferior alveolar process. Advanced
58	56	30.8	18.9			0.113	Carcinoma superior alveolar process. Very malignant
59	42	23.8	13.3	3.2	5.3	0.115	Carcinoma inferior alveolar process. Advanced. Slough
60	60	34.6	15.4	4.2		0.099	Carcinoma superior alveolar process. Not very malignant
		34.3	16.4		6.0	0.110	Same case, 2 months later
61	56	30.8	15.4		6.3	0.145	Carcinoma superior alveolar process. Rapidly growing
62	63	40.7	19.6		8.2	0.109	Carcinoma floor mouth. Degenerative changes of age. Local, but not very malignant
63	35	30.8	15.0		6.0	0.084	Carcinoma inferior alveolar process. General condition fair. Advanced, but local
64	70	29.4	16.8		7.1	0.120	Floor mouth; local. Nephritis. Alcoholic

TABLE 1—Continued

NUMBER	AGE	NON-PROTEIN NITROGEN	UREA NITROGEN	URIC ACID	AMINO ACID NITROGEN	SUGAR	REMARKS
--------	-----	-------------------------	------------------	-----------	------------------------	-------	---------

<i>Mouth—Continued</i>							
		mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	per cent	
65	58	25.9	13.6		7.9	0.102	Carcinoma inferior maxilla. Rapid and very malignant
66	63	27.3	17.5	<1		0.107	Carcinoma inferior alveolus. Rapid and very malignant
67	40	35.1	13.3		10.5	0.111	Carcinoma superior alveolus. Rapid and advanced
68	54	19.1	10.5		7.2	0.126	Floor mouth. Long duration. Far advanced. General con- dition bad
69	54	28.0	14.0				Early antrum. Rheumatism
70	75	37.9		3.0			Papillary carcinoma of buccal cavity. Senile changes
71	52	28.7	11.5	<1			Carcinoma superior maxilla. Local and slowly growing
72	53	36.5		2.3			Carcinoma floor mouth. Short and very malignant

<i>Prostate</i>							
73	66	29.4	17.5			0.095	Long duration. Locally, far ad- vanced. Alcoholic
74	66	32.2	19.6			0.100	Postoperative. Recurrence
75	69	30.8	14.0	3.1	5.9	0.137	Terminal stage. Chronic rheu- matism
76	71	26.6	10.5	3.7		0.133	Rapid course
77	51	28.0	15.4		7.2	0.085	Rapid and very malignant
78	62	43.4	28.7	5.1	6.6	0.105	Short duration. Very malig- nant. Metastases. Kidneys
79	74	31.5	15.4		7.8	0.112	Long duration
80	78	28.0	14.3		5.5	0.125	Rapid and very malignant
81	72	44.2	21.0		8.4	0.105	Local and of long duration. Pyelitis
82	33	23.8	12.8			0.100	Extensive and very malignant. Sarcoma

<i>Rectum</i>							
83	66	32.2	16.8	2.5	8.9	0.126	Rapid. Well advanced
84	46	22.4	9.8	1.5	7.6	0.084	Recurrent. Very malignant
85	39	28.7	12.3		8.7	0.105	Well localized. Tr. alb.

TABLE 1—*Continued*

NUMBER	AGE	NON-PROTEIN NITROGEN	UREA NITROGEN	URIC ACID	AMINO ACID NITROGEN	SUGAR	REMARKS
--------	-----	-------------------------	------------------	-----------	------------------------	-------	---------

<i>Rectum—Continued</i>							
		mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	per cent	
86	37	30.8	15.4	2.4	9.0	0.090	Far advanced. Very malignant
87	40	30.8	14.0		7.8	0.149	Recurrent; slow growing; extensive
88	68	30.8	16.1		8.0	0.101	Local lesion. Well advanced
89	60	29.4	16.4		8.4	0.117	Recurrent. Long duration.
90	54	18.2	9.7			0.084	Very malignant
91	62	29.4		<1			Postoperative. No visible carcinoma
92	52	31.5		1.8			Extensive. Malignant. Immunity
93	49	32.2		1.6	10.7		Short duration. Very malignant. Advanced
94	50	26.6	14.0	<1			Adenocarcinoma. Local lesion, not far advanced
95	50	35.1		2.6			Long duration. Slow growing

<i>Bladder</i>							
96	62	30.8	17.9			0.100	Papillary. Short duration. Rapid growth
97	71	37.9	24.5	1.3	10.4	0.118	Advanced. Degenerative changes
98	71	30.8	16.8	1.7	5.6	0.072	Rapid course. Extensive lesion
99	31	23.1	9.8		6.2	0.118	Advanced. Very malignant
100	53	33.6	15.4	4.4	6.9	0.094	Well advanced
101	63	37.9	24.5	3.5	7.0	0.125	1 year duration. Kidneys

<i>Melanoma</i>							
102	46	33.6		5.1		0.062	Terminal stage. Primary mole two years previous
103	35	29.4	14.0		8.6	0.111	Chest
104	58	30.1	17.5	4.5		0.112	Palate. Far advanced. Long duration

<i>Sarcoma, soft</i>							
105	34	23.8	15.4			0.095	Pleura. Advanced. Immunity, Reacts to x-ray

TABLE I—Continued

NUMBER	AGE	NON-PROTEIN NITROGEN	UREA NITROGEN	URIC ACID	AMINO ACID NITROGEN	SUGAR	REMARKS
--------	-----	-------------------------	------------------	-----------	------------------------	-------	---------

<i>Sarcoma, soft—Continued</i>							
		mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	per cent	
106	61	30.1	12.9	1.8		0.091	Breast. Metastases to eye after 6 years
107	55	30.8	15.4		7.6	0.084	Testis. Advanced. Short duration
108	33	32.2	19.3	3.3	5.4	0.120	Abdominal wall. Albumen
109	47	26.6	13.3	3.2		0.110	Nasopharynx
110	30	35.1		1.8			Teratoma testis
111	56	25.2	10.5	1.8		0.112	Advanced
112	48	32.2	17.5			0.118	Choroid, locally advanced
113	67	30.1	16.8				Back. Local. Operable
114	52	30.1	15.0	<1			Testis. Anaplastic
115	47	24.5	11.9			0.115	Rapid and malignant

<i>Lymphosarcoma</i>							
		mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	per cent	
116	47	30.1	13.3		4.7	0.120	Responded to treatment and then rapidly progressed
117	50	27.2	15.7		6.2	0.110	Rapid; died in 2 months
118	35	30.8	18.2	5.0			Bulky; not far advanced. Kidneys
119	52	35.1	15.4			0.070	Recurrence

<i>Sarcoma, hard</i>							
		mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	per cent	
120	21	39.3				0.091	Femur. Advanced. Metastases in chest
121	44	37.9	23.8			0.104	Humerus. Terminal. Metastases in lung
122	39	21.0	12.9			0.107	Humerus. Myxoma. Very rapid
123	55	28.7	12.6	1.7		0.103	Fascial thigh. Very malignant. Sepsis
124	24	35.1	22.9		7.7	0.096	Occipital. Terminal stage
125	44	21.0	10.1	1.9	5.2	0.102	Atypical. Arm. Metastases in lung. Advanced
126	18	28.0	13.3		8.3	0.096	Recurrent. Toxins. Marked immunity

TABLE 1—Continued.

NUMBER	AGE	NON-PROTEIN NITROGEN	UREA NITROGEN	URIC ACID	AMINO ACID NITROGEN	SUGAR	REMARKS
<i>Sarcoma, hard—Continued</i>							
		mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	per cent	
127	34	31.5	14.7			0.144	Tibia. Long duration. Far advanced. Toxins
128	33	27.3	13.6		10.4	0.117	Myeloma humerus
129	20	26.6	12.6		6.5	0.117	Femur. Extensive. Long duration
130	18	24.5		<1	9.6		Superior maxilla. Recurrent. Toxins
131	18	22.4	11.2	1.8			Rib. Metastases
132	38	25.9		1.4			Tibia. Metastases to chest
133		23.8	12.2			0.112	Pelvis
<i>Neck</i>							
134	65	30.6	12.3			0.146	Far advanced. Degenerative changes
135	22	28.0	13.3			0.099	Rapid. Very malignant sarcoma
136	33	26.6	12.1		8.4	0.117	Slow; extensive locally
<i>Tongue</i>							
137	42	28.0				0.087	Very malignant. Syphilis
138	45	35.1	16.8			0.142	Very malignant. Kidneys
139	57	22.4	9.8		5.8	0.144	Very malignant. Extensive recurrence
140	63	30.1	14.0	4.2	7.7	0.090	Extensive lesion. Kidneys
141	58	24.5	12.2		3.2	0.116	Early lesion. Syphilis
142	48	30.1		2.2			Early lesion. Good immunity
143	48	23.1	12.6		7.6	0.123	Late and advanced. Very malignant
<i>Larynx</i>							
144	50	33.6				0.130	Advanced lesion. Diabetes
145	38	28.7	19.6	2.1		0.084	Long duration; no generalization
146	25	25.6	10.5		7.6	0.093	Long duration; no generalization
147	40	35.1	22.4	2.5		0.090	Long duration; far advanced; starvation
148	50	29.4		<1	10.3		Short duration. Very malignant

TABLE I—Continued

NUMBER	AGE	NON-PROTEIN NITROGEN	UREA NITROGEN	URIC ACID	AMINO ACID NITROGEN	SUGAR	REMARKS
<i>Lip</i>							
		mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	per cent	
149	70	33.6	17.6			0.097	Slow growing. Recurrent. Arteriosclerosis
150	65	30.8	14.7	3.0	7.4	0.086	Terminal stage
151	44	30.8	17.1		6.0	0.120	Localized. Early growth
152	65	35.8		1.9			Recurrent. Arterial changes
153	61	37.2		2.7			Local. Not advanced
154	56	28.7			12.2		Local. Not much infiltration
<i>Stomach</i>							
155	59	30.8	11.2			0.110	Far advanced
156	46	21.0	10.5	2.4	10.0	0.090	Advanced; rapid. No starvation
157	46	35.1	11.5		6.9	0.100	Advanced; rapid
158	48	40.4	22.1			0.103	Advanced; long duration. Negative urine
159	66	28.0	19.6	3.3	8.0	0.089	Advanced. Starvation
160	72	39.3		4.6			Moderately advanced
161	60	24.5		1.0			Long duration. Starvation
162	48	28.0	18.2				Short duration. Starvation
163	54	42.1	18.6	3.6			Wellnourished. Duration 2 years
164	55	35.8	21.0	2.9	5.3	0.367	Long duration. Diabetes
165	50	148.8	119.3	6.0		0.153	Kidneys. Carcinoma stomach?
166	50	171.2		5.9			Kidneys. Carcinoma stomach?
<i>Esophagus</i>							
167	60	35.1	19.6	1.7		0.116	Long duration; localized
168	45	32.2	19.3		8.4	0.113	Long duration. Slow growing. Kidneys
169	53	30.1	16.4		6.7	0.120	Local. Well advanced
170	72				7.2	0.090	Starvation
171	52	30.8		1.7			Early lesion
<i>Hodgkins</i>							
172	50	39.3	26.6	5.7		0.101	Advanced nephritis
173	24	26.6	12.6		6.9	0.103	Axillary. Bulky lesion
174	36	25.2	12.2		8.4	0.095	Extensive. Duration 1 year
175	22	28.0	18.2		7.4	0.101	Advanced. Good general condition

TABLE I—*Concluded*

NUMBER	AGE	NON-PROTEIN NITROGEN	UREA NITROGEN	URIC ACID	AMINO ACID NITROGEN	SUGAR	REMARKS
<i>Tonsil</i>							
		<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>per cent</i>	
176	50	29.4	11.5			0.090	Terminal stage
177	50	30.1	15.8		8.2	0.099	Long duration. Good general condition. Immunity. Nephritis
178	51	28.7			8.8		Moderately extensive. Alcoholic
<i>Miscellaneous</i>							
179	46	37.9	15.5	4.2	10.3	0.070	Chronic lymphatic leukemia. Negative urine. White count 308,000
	46	37.9	23.1	2.5	9.2	0.100	Same case in 2 weeks. x-ray. White count, 136,000
180	37	50.5	23.1	5.8	18.4	0.105	Myelogenous leukemia. Negative urine. White count, 245,000
	37	50.5	31.5		9.9	0.125	Same case in 2 weeks. Radium. White count, 89,000
181	47	33.6	13.3		6.9	0.121	Lymphatic leukemia. White count, 245,000
182	59	47.7	21.0	3.2	14.9		Polycythemia; red cells, 7,872,000; hemoglobin, 105; white cells, 57,950
183	68	29.4	19.6			0.099	Carcinoma thyroid. Recurrent. Long duration
184	57	29.4	15.4	2.8	5.6	0.111	Epithelioma buttocks. Advanced. Very malignant
185	45	30.8	21.7	1.9		0.082	Mixed tumor of parotid. Not very malignant
186	59	28.0	13.6			0.090	Carcinoma thyroid. Metastases to stomach
187	27	33.6	17.5		7.8	0.135	Abscess of brain
188	50	29.4		<1	11.6		Carcinoma of pancreas. Good resistance
189	39	36.5		1.1			Brain tumor

and it is quite to be expected that these cases should show the most marked chemical variation from the normal. It is of interest in this connection to note that Folin (17) has reported abnormally low nitrogen figures for the blood of normal pregnant women. Dr. Wm. G. Lyle (private communication) has also noted very low nitrogen figures in the blood of pregnant women in investigations carried out at the Roosevelt Hospital. Cancer and pregnancy are related in that both involve rapid cell proliferation within the body, and it is of much interest to note the chemical similarity of the blood in the two conditions.¹ Mosenthal and Lewis (18) report a low urea value for 5 cases of anemia.

Looking at the 189 cases as a whole, we find that 23 per cent have a non-protein nitrogen content of 25 mgm. per 100 cc. or below, and only 31 per cent are above 30 mgm. Urea nitrogen is also low, 31 per cent of the cases having 12 mgm. or less per 100 cc. of blood. Fifty-five per cent of the cases have a urea nitrogen representing less than 50 per cent of the total non-protein nitrogen. Uric acid values appear to be normal in our cases, except when kidney complications are present. Exceptions to this statement are to be noted in two cases of melanoma in which the uric acid figures are high, although there were no kidney complications detectable. Amino acids are present in slightly increased quantity, as compared with the normal figures reported by Bock. The sugar values are practically normal, or at least our cases as a whole would average within normal limits. This conclusion is contrary to the findings of several previous investigators, but we believe that our longer series of cases demonstrates that where there is no kidney involvement or diabetes present the blood sugar figures in cancer are not abnormally high. Certainly there is no relationship between the severity of the disease and an increased blood sugar.

¹ Dr. A. F. Coca has found almost complete absence of urea from the tissue of some breast tumors which he examined. (Private communication.)

PATHOLOGICAL AND CLINICAL FACTORS

In the earlier portion of this paper we have called attention to the fact that interpretations of the chemical data obtained from the blood of cancer cases should not be made without reference to the age and general condition of the patient. It is also necessary to consider the location of the tumor or tumors, as well as special involvement of any organ due to other disease or to the degenerative changes of advancing age. We believe that these factors have been largely neglected in such studies as have hitherto been reported. In tables 2, 3, and 4 we have presented our findings in such a way as to try to bring out any relationship which might exist between the results of the chemical analyses and the various factors above enumerated. A brief discussion of the results of such classification follows.

TABLE 2
Average results according to age

NUMBER OF CASES	DECADE	NON-PROTEIN NITROGEN	NUMBER OF CASES	DECADE	NON-PROTEIN NITROGEN
11	20-30	27	46	50-60	30
26	30-40	26	30	60-70	33
45	40-50	29	10	70-80	33

Age. In table 2, 168 patients are classified with age as the sole basis of the classification, and the average figures obtained for the non-protein nitrogen are recorded for each decade. From this table it appears that in the period from twenty to forty years, age has no influence upon the non-protein nitrogen content of the blood. For the following decades up to seventy years there is a slight but steady increase in this nitrogen value. It is to be expected that the degenerative processes of age, which are so apt to involve the kidneys, even where such changes may not be detectable clinically, would influence the non-protein nitrogen of the blood so that this figure would be higher than for younger individuals. Whether figures similar to ours would be found in supposedly strictly normal individuals of like ages cannot be

TABLE 3
Averages according to age and location of tumor

NUMBER	AGE	NON-PROTEIN NITROGEN	UREA NITRO- GEN	URIC ACID	AMINO ACID NITROGEN	SUGAR	UREA NITRO- GEN	AMINO ACID NITROGEN	LOCATION OF DISEASE
		mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	per cent	per cent	per cent	
1-40	45	26.6	13.6	2.6	7.8	0.105	51.0	29.6	Uterus*
41-53	45	27.4	13.1	2.9	7.7	0.105	47.6	26.8	Breast
54-72	58	33.6	15.5	3.2	6.1	0.111	46.0	18.0	Mouth
73-82	67	31.8	16.9	4.6	6.9	0.109	53.2	21.8	Prostate
83-95	51	29.1	13.8	2.1	8.6	0.107	47.4	29.6	Rectum
96-101	58	32.4	18.6	2.7	7.8	0.104	57.5	24.0	Bladder
102-104	31	31.1	15.7	4.8	8.6	0.095	59.5	27.6	Melanoma
105-115	48	29.9	15.3	2.4	6.5	0.093	51.0	21.8	Sarcoma, soft
116-119	46	30.8	15.7	5(1)	5.5	0.100	51.0	18.6	Lymphosarcoma
120-133	30	28.1	14.5	2.3	8.1	0.108	51.6	28.9	Sarcoma, hard
134-136	40	28.4	12.6		8.4	0.120	44.5	29.6	Neck
137-143	51	27.6	13.1	3.2	7.0	0.117	46.5	28.8	Tongue
144-148	40	30.5	17.5	2.3	8.9	0.099	57.3	29.4	Larynx
149-154	60	32.8	16.5	2.5	8.5	0.098	50.4	25.9	Lip
155-166	56	32.5	16.6	3.0	7.8	0.098	51.0	24.0	Stomach*
167-171	56	32.1	18.4	1.7	7.4	0.109	57.4	23.3	Esophagus
172-175	33	26.6	14.3		7.6	0.094	47.6	26.8	Hodgkin's†
176-178	50	29.5	13.7		8.5	0.094	46.5	28.8	Tonsil

* Excluding 2 cases.

† Excluding one case.

TABLE 4

NUMBER	AGE	NON-PROTEIN NITROGEN	UREA NITROGEN	URIC ACID	AMINO ACID NITROGEN	SUGAR	UREA NITROGEN	AMINO ACID NITROGEN	LOCATION OF DISEASE
		mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	per cent	per cent	per cent	
8	40	24.6	11.7	2.4	7.6	0.095	47.5	31.0	Uterus
6	39	25.2	11.5	2.9	7.1	0.107	46.0	27.8	Breast
7	56	30.8	16.1		8.2	0.117	52.1	26.8	Mouth
4	58	26.6	13.1	3.7	6.3	0.100	49.3	23.9	Prostate
5	52	26.6	12.7	1.8	8.3	0.092	47.6	31.4	Rectum
3	54	28.3	14.8	1.5	5.9	0.096	52.4	21.0	Bladder
1	47	24.5	11.9			0.115	48.0		Sarcoma, soft
2	47	24.4	12.8	1.7		0.106	52.0		Sarcoma, hard

answered without far more extensive studies on such persons than are so far available.

In table 3, the average results of the chemical analyses are given in connection with the average age of the patient and the location of the tumors. Table 4 represents the average results in the particularly rapidly growing tumors according to the different organs affected. From these tables we find that the non-protein nitrogen values in uterine, breast, and tongue cancers are lowest, while mouth, stomach, esophagus, and bladder tumors show the highest values. In the case of each organ, numerous factors of importance in the interpretation of the results should be mentioned.

Uterus. In 40 cases of uterine cancers the average age is forty-five years. This is within a decade in which average non-protein nitrogen value of cancer cases as shown in table 2 is 29 mgm. per 100 cc. The average non-protein nitrogen value for the uterine cases is 26.6 mgm. per 100 cc. In this decade degenerative changes are in general a negligible factor, although pyelitis and kidney changes may often be found in the terminal period of the disease from bladder and ureteral involvement. Uterine cancer is a very malignant growth and often rapid in its progress. Distant metastases, however, are comparatively infrequent. The majority of the patients were in an advanced stage of the disease when the analyses were made. Case 36 (table 1) is of interest in this connection. The growth in this case was apparently limited to the cervix in a patient thirty-eight years of age and the non-protein nitrogen was only 16.8 mgm. per 100 cc. Complete retrogression and apparent cure of the lesion resulted from the application of radium. No other pathological conditions were found, except a moderate anemia. Blood analysis recently made in this case gives the high non-protein nitrogen values of 34.5 mgm.

Breast. In 13 patients with mammary cancer the average age is the same as in the uterine cases. The non-protein nitrogen value for this series is 27.4 mgm. per 100 cc. Most of these lesions were advanced, distant metastases were common, and in many the progress was very rapid.

Tongue. The average age in six cases of carcinoma of the tongue is fifty-one years. The decade non-protein nitrogen value is 30 mgm. per 100 cc. Syphilis and degenerative changes are common. These lesions of the tongue were extensive and perhaps more malignant than cancer in any other organ.

Mouth. (Exclusive of lip and tongue tumors.) Eighteen mouth cases have an average age of fifty-eight years with a decade non-protein nitrogen of 30 mgm. per 100 cc. The average non-protein nitrogen content is 33.6 mgm. per 100 cc. Alcohol, syphilis, and teeth infections made nephritis and diverse degenerative changes sufficiently frequent to explain the high non-protein nitrogen value.

In cancer of the *prostate* and *bladder* the average age is sixty-seven and fifty-eight years respectively and the average non-protein nitrogen 31.8 mgm. and 32.6 mgm. per 100 cc. The location of the disease, as well as the comparatively advanced age of these cases, makes pyelitis and kidney changes a frequent factor in the results of the blood analyses.

Stomach. In 11 cases of carcinoma of the stomach the average age is fifty-eight years. The decade non-protein nitrogen value for this age is 30 mgm. and the average non-protein nitrogen is 32.5 mgm. per 100 cc. All of the cases except two were far advanced. In two cases there is a very high non-protein nitrogen.

Rectum. The average non-protein nitrogen value in seven rectal cases is only a trifle less than the decade value. In all of the cases the progress of the disease was slow.

Sarcoma. Twenty-three cases of sarcoma of hard and soft tissues are all very malignant and rapidly-growing. The non-protein nitrogen value is 28.1 mgm. and 29.9 mgm. per 100 cc. respectively.

Lymphosarcoma. In three cases of lymphosarcoma, with an average age of forty-six years, the course of the disease was rapid. The non-protein nitrogen value is slightly higher than the decade value.

CONCLUSIONS

1. Non-protein nitrogen and urea nitrogen are in general low in the blood of cancer patients. Amino acid nitrogen is slightly above normal. Low results are not so obvious when other pathological conditions coexist.

2. Non-protein nitrogen and urea nitrogen of blood are consistently low in clinically malignant cases.

3. Uric acid, except in cases with kidney complications and in two cases of melanoma, is not abnormal in cancer blood.

4. Blood sugar is not generally increased in cancer. Twenty-six per cent (diabetics and nephritics excluded) show a figure somewhat above the normal and 13 per cent show a figure below normal.

REFERENCES

- (1) FREUND: *Wien. med. Bl.*, 1885, viii, 268.
- (2) TRINKLER: *Centralbl. f. d. Wissensch.*, 1890, xxviii, 498.
- (3) MATRAI: *Pest. med.-chir. Presse*, 1885, xxi, 629.
- (4) LEWIS AND BENEDICT: *Med. Rec.*, New York, 1914, lxxxvi, 650.
- (5) MENTEN: *Jour. Cancer Research*, 1917, ii, 179.
- (6) LUDEN: *Jour. Lab. and Clin. Med.*, 1916, i, 662.
- (7) AUTENRIETH AND FUNK: *München. med. Wehnschr.*, 1913, lx, 1243.
- (8) BLOOR: *Jour. Biol. Chem.*, 1915, xxiii, 317.
- (9) DENIS: *Jour. Biol. Chem.*, 1917, xxix, 93.
- (10) GREENWALD: *Jour. Biol. Chem.*, 1915, xxi, 61.
- (11) BOCK AND BENEDICT: *Jour. Biol. Chem.*, 1915, xx, 47.
- (12) MARSHALL: *Jour. Biol. Chem.*, 1913, xv, 487.
- (13) BENEDICT: *Jour. Biol. Chem.*, 1915, xx, 629.
- (14) NEUWIRTH: *Jour. Biol. Chem.*, 1917, xxix, 477.
- (15) BOCK: *Jour. Biol. Chem.*, 1917, xxviii, 357.
- (16) LEWIS AND BENEDICT: *Jour. Biol. Chem.*, 1915, xx, 61.
- (17) FOLIN: *Jour. Am. Med. Assn.*, 1917, lxix, 1209.
- (18) MOSENTHAL AND LEWIS: *Jour. Am. Med. Assn.*, 1916, lxxvii, 933.

ON SPIROPTERA CARCINOMATA AND THEIR RELATION TO TRUE MALIGNANT TUMORS; WITH SOME REMARKS ON CANCER AGE

JOHANNES FIBIGER

From the Anatomo-Pathological Institute of the University of Copenhagen

Received for publication, July 25, 1919

In this Journal (1918, iii, 227) Bullock and Rohdenburg have criticized some reports published during the last years, concerning the experimental production of malignant tumors (Fibiger, Yamagiwa and Ichikawa) and tumor-like formations (Stahr). Bullock and Rohdenburg emphasize that

An analysis of the several reports mentioned reveals a number of assertions common to each, which are at variance with the recognized laws of malignant tumor growth, while, at the same time, it brings out points which cast a certain amount of doubt upon the authors' interpretation of their results.

In the following I shall reply to Bullock and Rohdenburg's criticism, though only in so far as it concerns the investigations on *Spiroptera carcinoma* communicated by me. I can so much the less avoid occupying myself with the assertions of these authors as their untenability seems to some extent to be due to an insufficient acquaintance with or a misapprehension of the details of my investigations of 1913 and 1914 (1-4). My most recently concluded investigations (8-11), which corroborate the results of the former, were not published until after the appearance of the authors' paper and so could not be known to them.

Bullock and Rohdenburg base part of their criticism on a comparison between the result of my investigations on *Spiroptera carcinoma* and the result of investigations carried out by them-

selves on the effect of mechanical, chemical, or mechanico-chemical irritants¹ on the stomach of the rat.

As a result of the investigations Bullock and Rohdenburg, in several passages as also in the summary of their paper emphasize:

1. That "Chemical or mechanico-chemical irritation of the squamous epithelium of the rat's stomach results in localized papillary tumors suggesting the canceroid growths produced by nematode worms."

Against this it must be pointed out that none of the figures accompanying the authors' paper has any real likeness either to epitheliomata (canceroids, carcinomata) in general or to the *Spiroptera carcinomata* produced by me and given in my plates, but only shows changes that cannot be designated as anything but simple hyperplasia and hyperkeratosis of the squamous epithelium of the fundus of the stomach of the rat with down-growth of the epithelium, combined with slight papillary transformations of the mucosa. Nor is it evidenced by the text that the authors have observed changes of genuine carcinomatous type.

On the other hand I will not deny that the changes produced by the authors accord with non-carcinomatous changes of similar kind produced almost constantly by the invasion of *Spiroptera neoplastica* (*Gongylonema neoplasticum*) (5) in the fundus of the stomach of the rat, disregarding the fact that these latter changes are often more pronounced. That changes of this kind are the usual effect of *Spiroptera* infection on the fundus of the stomach in rats and mice has been described in detail in my papers of 1913 and 1914 (1-4) the first of which contained numerous photographs showing what these changes were like. In all the 111 rats dealt with in these papers, in which the fundus of the stomach was at all pathologically transformed, such changes were demonstrated, and in 92 out of the 111 rats the changes were only of this type.

But it must be sharply emphasized that changes of the character here mentioned (which I have also had occasion to observe

¹ The irritants employed were spines of pig bristles, pin points, Scharlach R-oil, pine tar, pine tar oil (see the original paper).

may be produced from other causes than Spiroptera) have not in a single case been looked upon by me as carcinoma-like or as true carcinomata, but have been regarded exclusively as mentioned above, i.e., only as hyperplasia, hyperkeratosis, benignant down-growth and heterotopy of the epithelium, combined with inflammation and frequently also with papillary transformation of the mucous membrane.

If in my investigations I had only been able to produce changes of this category any talk of a real Spiroptera carcinoma would have been entirely excluded.

When, however, it can now as little as hitherto be doubted that real carcinomata, morphologically possessing the specific characters of malignant epitheliomata, can be produced by Spiroptera infection, this is due to the observation of phenomena of an entirely different kind.

As will be seen *inter alia* from detailed descriptions in my previous papers from 1913 and 1914 (1-4) in a considerable number of Spiroptera infected rats, together with the above mentioned simple epithelial hyperplasia, more or less pronounced epithelial changes of quite another type occur, and this type has the specific character of the malignant squamous-celled carcinoma in pronounced shape. Changes of this kind were found in 19 out of the above mentioned 111 rats, combined with simple hyperplasia, and in later investigations (8) concluded in 1918, I have further demonstrated such real carcinomatous changes in 65 rats.

In order to throw light upon the peculiarities which characterize these changes and which entitle us to regard them as true carcinomata I shall quote as follows from one of my latest papers (1918) (8):

I have only felt entitled to fix the diagnosis of carcinoma in such cases as exhibited—through a series of sections—the following changes:

1. Heterotopical downgrowth of epithelial cells belonging not only to the normal type of the basal epithelial layers, but mixed with atypical and keratinized cells in abundance partly arranged as spherical masses and horny globes.

2. Infiltrative growth of these heterotopical and atypical epithelial cells into deeper layers, splitting up invasively the elements of the connective tissue of mucosa and the muscle cells of the muscularis mucosæ, forming isles and spurs in the latter or—as most frequently seen—also penetrating through this membrane into the superficial or deeper layers of submucosa.

And that these changes described by me as genuine carcinoma show the very closest agreement with the well-known changes in fully pronounced and typical squamous-celled carcinomata (malignant epitheliomata) in man and animals will be further evidenced by the plates accompanying my papers (see e.g., in my paper of 1913 (2), plates XI–XV, figs. 73, 78, 79, 83–85; in my paper of 1914 (4), figs. 15, 16, and others; also photographs in my papers of 1918 (8)).

What has been here adduced will sufficiently demonstrate that in reality—in opposition to the opinion of Bullock and Rohdenburg—there exists no similarity, but on the contrary, considerable divergence, between the hyperplasia produced by these experimenters and the changes characterized by me as real carcinoma, which in Spiroptera infected rats (frequently in black and white rats) occur together with epithelial hyperplasia, hyperkeratosis, downgrowth of the epithelium, and papillary transformation. These latter changes, which show considerable likeness to those produced by Bullock and Rohdenburg, must be sharply distinguished from the really carcinomatous changes, and, as already mentioned, have never been regarded by me as carcinoma-like or really carcinomatous.

To what degree I look upon the simple benignant hyperplasia as different from the real carcinoma is evidenced by my latest papers (of 1918) (10). Referring to the arguments there adduced, I shall here restrict myself to emphasizing that I regard the two forms of change, the simple epithelial hyperplasia and the real carcinoma occurring side by side in the fundus of the stomach in Spiroptera infected rats, not only as being morphologically but also as fundamentally different, mutually independent, simultaneously occurring effects of the same etiological factor, the Spiroptera infection.

The Spiroptera infection in all kinds of rats and mice hitherto examined by me produces inflammation, epithelial hyperplasia, hyperkeratosis, downgrowth of the epithelium, and papillary changes in the squamous-cell part of the stomach. Together with these more or less extensive changes, pronounced carcinomatous processes were observed in black and white rats in more than 50 per cent of the experimental animals. In wild house rats I have hitherto observed carcinomatous changes in only a single case, in white mice only in 3 cases. In wild Norway rats, house mice, and forest mice, I have not as yet observed carcinoma, though epithelial hyperplasia and downgrowth in these animals often reaches an enormous development, exceeding what may as a rule be observed in black and white rats. The investigations on the effect of the Spiroptera on the various rodents have, however, not yet been concluded, and only as regards the black and white rats and the white mice comprise a considerable number of animals.

2. Bullock and Rohdenburg further point out that the Spiroptera carcinoma in the fundus of the stomach of the rat does not invade the muscularis or the glandular portion of the organ.

As a matter of course this circumstance does not really involve any objection whatever to the diagnosis of carcinoma, granting that the observed changes otherwise possess the fully typical properties of the carcinoma. Like all other carcinomata, the Spiroptera carcinoma requires a certain time to spread ad maximum, and in a greater number of the cases observed by me the explanation why the muscularis had not been invaded and the process had not penetrated into the glandular portion must simply be sought in the fact that the animals died so early that the process had not yet reached its greatest extension.

This explanation is quite satisfactory in all the cases where the Spiroptera infected rats died one and one-half to three months or less after transmission of the Spiroptera, and the Spiroptera carcinoma had not extended to the deep layers of submucosa. But not even in other cases, where the rats had survived the transmission of the Spiroptera for a longer time (maximum eight to nine months), and the carcinoma had invaded large areas of

the deepest layers of submucosa, did I ever find the muscularis carcinomatous, not even in cases where the carcinoma had produced metastases.

The reason for this might, of course, be found in these cases also in the fact that the rats concerned had succumbed so soon to the Spiroptera affection or complicating diseases that the carcinoma in the fundus of the stomach had not yet reached its greatest development. For it must be recollected that the Spiroptera affection, whether it consists only of papillary transformation of the fundus, or whether this change is further complicated by carcinoma, is in any case an exceedingly serious disease, which is bound to weaken the nutrition and digestion of the rats and to a great extent shorten their span of life.

Now it cannot be doubted that black and white rats, which have chiefly been employed in the investigations, are but little resistant, whether the Spiroptera affection of the fundus of the stomach is carcinomatous or not, since they are not able to survive the infection for such long periods as other rats or mice, but succumb comparatively early; sometimes, it would seem, from the Spiroptera disease alone, but frequently also from infectious complications (especially pneumonias). But of Spiroptera infected wild Norway rats, house rats, house mice, and white mice, not only a large number have proved able to survive the infection for a longer time, but many Spiroptera infected animals of these species have been able to live much longer than the average period for black and white rats.

For the elucidation of the question, whether the escape of the muscularis in carcinoma of the stomach in Spiroptera infected black and white rats should be sought in their shorter term of life, it will therefore be of interest to inquire into the behaviour of the carcinoma in the more resistant species. As mentioned above I have hitherto only found Spiroptera carcinoma in the fundus of the stomach in one house rat and 3 white mice, and in 2 of these cases (in the house rat and in a white mouse) the downgrowth of the carcinoma was of comparatively slight extent. But the two other cases observed in mice offer greater interest; one of these will here be reported, whereas the other has been previously (11) communicated in detail.

Mouse I. Six weeks old albino male mouse, fed January 12, 1917, on muscles from the femora and prothorax of 3 cockroaches (*Periplaneta orientalis*) infected with Spiroptera. Upon examination of the excrements of the mouse, typical Spiroptera eggs were demonstrated on July 30, 1917. The mouse died February 26, 1918, about fifteen months old, 410 days after the transmission of the Spiroptera. Its weight at death was 15 grams.

Post mortem examination shows diffuse purulent peritonitis. The stomach measures rather more than 1 cm. from side to side and about 7 mm. from top to bottom. The fundus is greatly thickened and irregularly knotted, especially at the greater curvature. At the fore and top part of the latter, where the thickening is most pronounced, the cause of the peritonitis is found in a complete perforation of the wall at a projecting portion, near which the stomach adheres to the posterior surface of the liver.

The tongue and esophagus exhibit no special changes. The right lung contains a sharply marked-off white nodule, rather larger than a hemp seed, part of which is at once taken out, cut to pieces, and transplanted intraperitoneally in 10 young white mice weighing 8 to 10 grams. The organs otherwise normal and without metastases. The wall of the fundus shows papillary transformation in spots, especially in the perforated portion, the thickness of which is 4 to 5 mm. In the fundus, the epithelium is everywhere strongly desquamating and hyperplastic. Compact epithelial columns penetrate downwards here and there, piercing the submucosa which, like the connective tissue of the mucous membrane, shows more or less proliferation and infiltration with lymphocytes and leucocytes.

Over an area of about 4 mm., answering to the macroscopically demonstrated perforation, pronounced carcinomatous formation is found, atypical epithelial projections mixed with numerous epithelial pearls and horny globes penetrating heterotopically downwards and infiltratively invading the mucous membrane, submucosa, and muscularis. Both the inner and outer layers of this membrane are invaded by the carcinoma, which penetrates into the bundles of muscular fibrillæ, splitting these and extending further into the serosa, which shows advanced inflammation and adheres to the liver in a considerable area. The perforation has taken place through the central part of the carcinomatous portion, which shows strong necrosis of the carcinomatous masses. The surface epithelium everywhere, both in the carcinomatous part of the fundus and in the other portions, contains

numerous sections of *Spiroptera* as well as ripe eggs. The pyloric portion is normal. The adjacent lymph-nodes do not contain metastases. On the other hand, the nodule demonstrated in the right lung, as beforehand assumed, proves to be a metastasis built up of typical squamous-cell epithelium, keratinizing cells, and horny globes that fill out numerous alveoli. The bronchial epithelium is normal, nowhere metaplastically transformed, and has no connection with the metastasis. The metastasis is, however, over no small area in slight or pronounced necrosis, which may perhaps be the cause of the negative result of the transplantation experiments.

The case here communicated proves that the *Spiroptera* carcinoma in the fundus may very well invade not only the submucosa but also the muscularis and the other part of the stomach wall, and the other case previously communicated in detail, which I shall return to in the following, shows the same. In these two cases the experimental animals died respectively 410 and 482 days after the transmission of the *Spiroptera*. For comparison, it may be stated that the period of survival after transmission of the *Spiroptera* for carcinomatous black and white rats, at the utmost, and only for a smaller number of rats, has been 250 to 298 days. Hence the possibility cannot be ignored that the less deep penetration of the *Spiroptera* carcinoma into the stomach wall in black and white rats has—as mentioned above—only been a consequence of the fact that these rats have, on the whole, survived only for a short period the *Spiroptera* infection, and have therefore succumbed at a time when the carcinoma had not yet had sufficient time to penetrate into the muscularis.

But other factors also may possibly have played a part. The difference of the frequency of *Spiroptera* carcinoma in rats and mice pointed out in a preceding paper (10), indicates that differences not only according to individuals, but also according to species, and probably according to race, too, exist in the susceptibility to the cancer-producing power of the *Spiroptera*. Whether or not similar differences also exist in the power of resistance to the invasive growth of the carcinoma into the deepest layers of the wall of the fundus of the stomach, is a problem which cannot as yet be definitely solved.

That *Spiroptera carcinoma* possesses the power of invasive growth in muscular tissue will again appear from observations other than those mentioned above. As I have communicated in detail in a paper of 1918 (9), the *Spiroptera* invasion may also cause a development of carcinoma in the tongue of rats. The photographs of the 5 cases which accompany my paper, will show the occurrence of a very considerable, heterotopical, inter-muscular, carcinomatous downgrowth, which in two cases reached such a degree that the muscles of the tongue were invaded for one third or one half of their entire thickness. Metastases to lymph-nodes or to other organs could not be demonstrated.

But in two cases the carcinoma was found to invade the perineural lymphatic spaces, so that these latter were filled out to a considerable extent with typical carcinomatous cells, and with epithelial pearls and horny globes enclosing the nerves like a sheath.

3. Bullock and Rohdenburg furthermore emphasize that "the presence of metastatic growths remains after all unproved," and add that "one of the appearances" given in my photographs might be "a heterotopic epithelial area like that sometimes seen in the peritoneal nodes of man." I presume that the authors are here referring to a metastasis in a lymph-node described and photographed by me in my first paper (2).

With regard to this observation I submit the following statement: In a black and white rat the fundus of whose stomach contained a strongly developed *Spiroptera carcinoma*, in a retroperitoneal lymph-node 1.5 cm. long, 8 mm. broad, and 0.5 cm. thick, situated close to the spleen, two almost spherical, sharply marked-off, white, firm nodules were demonstrated, the diameter of which was about 1.25–1.5 mm. Upon examination in serial sections these nodules proved to be built up partly of epithelium of exactly the same type as the deeper layers of the carcinomatous cells in the fundus of the stomach, and, especially in the central part of the nodules, partly of strongly keratinizing flat epithelial cells. It was beyond all doubt that this was a typical carcinomatous metastasis in a lymph-node (see figures) (2) and it is entirely precluded that a "heterotopic epithelial area" should here be present. I should be greatly interested if the authors would give further details of the occurrence of

such "heterotopic" keratinizing squamous-cell areas in the lymph-nodes of rats. Personally—in spite of the examination through years of a very large number of peritoneal lymph-nodes—I have never observed phenomena of a similar character to those here mentioned either in man, rats, or mice.

Bullock and Rohdenburg further emphasize that I have not differentiated a pulmonary metastasis from a form of bronchopneumonia which has been described in rats and is characterized by the presence of a squamous epithelium through metaplasia of the bronchus. The authors speak only of "his pulmonary metastasis" as if I had only once observed phenomena of this kind, though in my paper (of 1914) (4) quoted by them, I give an account of two corresponding observations and my first paper (from 1913) (2) contains one. I suppose the authors refer to the observation communicated in this first paper (1913), about which I shall further state that, as, indeed, it appears from the accompanying photograph, distinct spherical nodules were present (greatest diameter about 0.8 mm.), well marked off from the bronchial epithelium, composed partly of closely packed masses of epithelial cells showing the same structure as that of the deeper layers of the carcinomatous cells in the stomach wall, partly of epithelial cells in which very perfect keratinisation had taken place; horny globes also were found. In serial sections neither was epithelial metaplasia discoverable in the bronchus and the broncheoli, nor did the surrounding areas of the parenchyma contain any bronchopneumonia. If in my earlier description of this observation I did not mention that metaplasia of the bronchial epithelium (as described, e.g., in mice by Tyzzer in 1909) might be precluded, the reason was, of course, that in the present case there could be no question at all of changes of such a nature. The lesion was a typical intravascular metastasis. Besides, the metastatic nature of these changes, as well as of the changes of the lymph-node mentioned above, has been generally recognized.²

² Preparations of these metastases have been shown at lectures in Copenhagen (The Medical Society, 1913), in Bruxelles (La troisième Conférence internationale pour l'étude du Cancer, 1913), in Berlin (Berliner medizinische Gesellschaft, 1913), in Kristiania (Meeting of Scandinavian Naturalists, 1916), and elsewhere.

That *Spiroptera* carcinoma may produce metastases in the lungs will appear not only from the preceding observation of *Spiroptera* carcinoma in a mouse, but from later observations in rats. By an exhaustive examination of both lungs in toto in serial sections in 5 white and black rats with primary carcinoma in the fundus of the stomach, I have found small nodules of a structure corresponding to that mentioned above, without it being possible to establish any metaplasia whatever of the bronchial epithelium. Nor has it ever been possible, in spite of the examination of lungs from numerous other, non-carcinomatous rats, to demonstrate changes showing a structure similar to these metastases, though in some few animals metaplasia of the bronchial epithelium was demonstrated. In all, metastases in the lungs have thus been observed in 8 cases. Furthermore, peritoneal metastases of a *Spiroptera* carcinoma in a mouse (II) will be described below.

Metastatic growths in the perineural lymphatic spaces have already been discussed.

4. As an objection to the *Spiroptera* carcinoma, Bullock and Rohdenburg point out that it is not stated definitely, "whether continuous irritation is necessary for continuous growth and whether a type of proliferation can be inaugurated that no longer depends upon the presence of the irritant."

With regard to this objection, I shall call attention to the fact that I have previously shown that the metastases of the *Spiroptera* carcinoma do not contain *Spiroptera*, and to the results of a series of investigations published in 1918 (10). In my first investigations (1913-14), I observed that as a rule several or numerous *Spiroptera* were discernible in stomachs which exhibited pronounced changes, while stomachs with slight or doubtful changes would harbour only a single or a few parasites. At the same time, however, I laid stress upon an exception to this rule; thus in certain cases the stomach will contain but a few nematodes in spite of pronounced carcinomatous changes, a fact which I ascribed to the original considerable number of *Spiroptera* having been diminished by a later emigration or dying off, analogous to the emigration of other species of nematodes, e.g., ascarides.

Renewed examinations, continued during the years that have passed since these original investigations, have shown that such an emigration plays a far more prominent part than I supposed at first. Referring the reader to one of my previous papers (1918) (10), in which all particulars are to be found, I shall only point out here that it is now beyond all doubt that in rats infected with the *Spiroptera* a considerable emigration of worms from the fundus of the stomach will take place in numerous cases when a period of three months or more has passed after the transmission of the worms. The number of *Spiroptera* in the stomach, even as early as three months after the transmission of a great number of the parasites (several hundreds), may be so reduced that the total epithelial covering contains altogether but one or two or a few parasites. This observation now becomes worthy of special interest by the fact that the frequency of carcinoma of the stomach among the longest lived rats—as will be seen in my paper (10)—proved to be about the same, no matter whether the stomach contained a great or a small number, or only one or two or a few worms.

Entirely corresponding observations can be made in mice. In mice which had survived the transmission of a great number of *Spiroptera* for a long time, I have often found the cardiac portion of the stomach to contain only a single or a very few *Spiroptera*, and in the advanced carcinoma of the stomach of a mouse (II), mentioned below, which died 482 days after transmission of the *Spiroptera*, no nematodes were macroscopically traceable by post mortem examination, and only by microscopical examination did I succeed in finding a single specimen. But that the fundus of the stomach had actually harbored egg-producing parasites for a period of at least eleven months was evidenced by the fact that the excrements of the mouse during life had contained *Spiroptera* eggs 322 days after the transmission of the worms. In this case the carcinoma had continued its vigorous development and produced large peritoneal metastases, in spite of the nearly complete disappearance of the *Spiroptera*.

Quite corresponding observations are also met with in Spiroptera infection of the tongue. Among the 5 rats, in the tongue of which Spiroptera carcinoma was produced, only 3 harbored the Spiroptera in the epithelium of their tongues, whereas the worms were entirely wanting in the tongues of the 2 longest lived of these rats, which had survived the transmission of the parasites from $5\frac{3}{4}$ to $6\frac{3}{4}$ months. And in the simultaneously developed carcinomata of the stomach of these 2 rats, only a very few specimens were to be found.

These observations show that the primary Spiroptera carcinoma in the fundus of the stomach of rats and mice and in the tongue of rats, as in the metastases, being once produced, is able to subsist and continue developing even if the irritant—viz., the Spiroptera—which originally induced it partly or completely disappears. As to the conclusions which may be drawn from these and other analogous observations, I shall refer the reader to my detailed report, and merely point out that the observations here mentioned prove that no significance can be attached to the objection of Bullock and Rohdenburg quoted above.

5. On the other hand, these authors are perfectly right in emphasizing that transplantation of the Spiroptera carcinoma has been unsuccessful in earlier experiments. It cannot, however, surprise any one that the experiments have given negative results if we consider: (1) that, as is generally known, great difficulties often occur in the propagation of strongly keratinizing tumors; (2) that, owing to technical difficulties, I may not, in all experiments in which I have been unable to inoculate any greater number of young rats, have succeeded in getting a sterile material for inoculation from the fundus of the stomach (or from the tongue); (3) furthermore, transplantation of metastases has been precluded, these secondary deposits having in most cases been of such tiny dimensions that only microscopical examination could establish their existence.

From the two mice mentioned above a suitable amount of metastatic deposit could be obtained for the first time. However, transplantation of the metastasis in the lung of mouse I gave no positive results. More successful were the transplan-

tations from mouse II mentioned above, of which only a short account will be given here, a complete record of the case having appeared in my special paper (11) in which further particulars are to be found.

Mouse II. Albino male mouse six to eight months old, fed on February 16, 1916, on muscles of 5 cockroaches (*P. americana*), infected with the Spiroptera. On January 3, 1917, typical Spiroptera eggs found in the excrements of the mouse. The mouse died on June 12, 1917, 482 days after the transmission of the Spiroptera. The weight at death was 24 grams.

The abdominal cavity is widely distended by an irregular tumor of the size of a large hazel nut, taking up the place of the stomach, and in the most lateral left portion adherent to the left lobe of the liver, to the left side of the diaphragm, and to the upper pole of the spleen. Tumor measures 2.5 x 2 x 1.5 cm. It seems due to an enormous new growth, partly in the stomach wall itself, partly perforating the latter, tumor masses being visible on the outer side of the stomach wall as well. The pyloric portion is apparently undamaged. The mouth cavity, tongue, and gullet are normal. In the mesentery, the omentum, and the peritoneal cavity, especially in the dorsal wall of the latter, numerous isolated, greyish-white, firm, roundish tumor nodules as large as the head of a pin up to the size of a pea are found. All the rest of the internal organs are normal; no metastases. No cutaneous or subcutaneous nodules. The wall of the fundus of the stomach proves to be about 0.5 to 1 cm. thick and consists of homogeneous greyish-white tumor tissue in which pronounced necrosis has taken place on the cavity side. The mucous membrane of the pyloric portion seems to exhibit no special changes; the line of demarcation towards the fundus is, however, hardly discernible. The stomach contains neither concretions nor hairballs. The intestines present no abnormalities.

On microscopic examination the greatly thickened part of the stomach wall is found to be the seat of a typical and extensively necrotic squamous-cell carcinoma with numerous areas of keratinisation. In most places the carcinoma extends to all the membranes of the wall, lining the cavity with a necrotic tissue and reaching not only into the serosa but penetrating this membrane and in great areas invading the adherent superficial parts of the liver, the spleen, and the diaphragm, the muscular bundles of which are split up by carcinomatous tissue.

In the wall of the fundus a single fully developed Spiroptera is found. The metastases all prove to be typical more or less keratinizing carcinoma, of the same structure as that of the cardiac portion.

Transplantation of the tumor was effected in immediate continuation of the post mortem examination. Fragments (weighing about 10 to 25 mgm.) of a peritoneal non-necrotic metastasis were used for the implantation. At the 1st transplantation, 12 white mice, weighing 10 to 15 grams, were inoculated, 7 subcutaneously and 5 intraperitoneally.

In 4 of the 7 mice inoculated subcutaneously, and in all mice inoculated intraperitoneally, tumors developed. The transplantations were continued, and in all the tumor proved transplantable during half a year for 4 generations. Taken together the transplantations gave positive results in 28 out of 55 inoculated mice which did not die immediately after the implantation. The tumor was preserved living by means of transplantation for altogether twelve months.

The inoculated particles very quickly reached a considerable size, weighing 9 to 11 grams, in several animals which died two to three months after the transplantation and being as large as a hazelnut, a walnut, or a plum.

Like the original tumor, the transplanted tumors exhibited the structure of a typical, keratinizing, squamous-cell carcinoma and kept their histological features almost unaltered through all 4 generations. They contained neither Spiroptera, parts of Spiroptera, nor eggs.

In several mice they grew invasively into the muscular tissue and into adjacent organs.

Again referring the reader to my recently published paper (11), in which the experiments here briefly summarized are communicated in extenso with all details and illustrations, I shall here restrict myself to emphasizing the main result of the experiments: the transplantability of the Spiroptera carcinoma.

6. Finally, Bullock and Rohdenburg allege as an essential argument against classing the Spiroptera carcinoma as a true malignant neoplasm, that the age of the animal is a negligible

factor in the incidence of these carcinomata, young animals being almost as susceptible as old ones.

In answer to this it must, however, be observed that no grounds for such an assertion are found in my paper of 1914 (4) quoted by the authors. In this it was merely pointed out that the *Spiroptera* carcinoma may also develop in quite young black and white rats, carcinoma in the fundus of the stomach having been demonstrated by me in a rat which, upon transmission of the *Spiroptera*, weighed only 30 grams, and at death (120 days later) 100 grams. The authors have, however, a greater justification for their conception of my opinion with regard to the occurrence of the *Spiroptera* carcinoma in young animals than can be gathered from my above mentioned paper, in so far as in later investigations (10) I have not been able to demonstrate with certainty a greater frequency of the carcinoma amongst unquestionably older than amongst unquestionably younger animals, and *Spiroptera* cancer occurred in rats which, both on the transmission of the *Spiroptera* and at death, were still young.

It has, however, proved impossible in these experiments to realize an exact estimation of the age of the rats and I have therefore felt bound to point out (10) only that the age of the animals is not likely to have been of any great importance.

But even if, through continued experiments, it should be established with certainty that *Spiroptera* carcinoma develops with the same frequency in young animals as in older ones, this cannot be advanced as an argument against the carcinomatous nature of this tumor. I do not agree with Bullock and Rohdenburg, either, when they declare "If we accept these tumors as malignant neoplasms, we must modify the current view concerning a cancer age, which has hitherto been held applicable to animals as well as to man;" or when they continue "We must conclude, in other words, that rats and rabbits differ in this respect from man and from the mouse, in which Murray, Slye, and others have shown that age is a very important factor for the origin of carcinoma."

The foundation for this generally accepted opinion here called attention to by the authors is, of course, the fact that carcino-

matous tumors in man and animals belonging to higher age periods are observed with a frequency which far exceeds the frequency of the relatively rare cases in which carcinoma can be demonstrated in young individuals.

It is, however, far too often forgotten that in determining the age at which man is attacked by carcinoma we can, as a rule, only take the date at which the carcinoma manifests itself as a demonstrable tumor; that this date is not tantamount to the real date of the first origin of the tumor is so obvious that any argument in support thereof is unnecessary.

This is of course also the case, and to a still greater degree with regard to the determination of the origin of tumors in animals. That tumors are almost exclusively found in older mice or rats really means only that tumors which have reached such a size that they are palpable or visible to the naked eye or on inspection with the lens are preeminently found in older animals; but it tells us nothing about the length of time in which these tumors have been present in non-demonstrable state, so that the real date of their earliest origin cannot be determined.

I must therefore dispute the correctness of the assertion advanced by Bullock and Rohdenburg, that investigations of tumors in mice and rats, however great the importance we must assign to them, have given more conclusive evidence of tumors preeminently occurring in older individuals than the evidence long since produced by clinical experience from man.

That the current opinion about the date of origin of the tumors is not often very far wrong must, however, be considered probable, as a considerable antedating of the origin of the tumors would only be justified on the improbable assumption that malignant tumors usually grow slowly.

But the rule that tumors occur preeminently in older individuals is not without exception, as is commonly known. Undoubtedly the number of true observations of malignant tumors in children has been greatly diminished through the critical examination of Philipp; still, a series of cases must be considered trustworthy. Certainly, incipient malignant tumors are sometimes found in young individuals dead from other diseases, but

it cannot be doubted that such tumors actually occur with greater frequency in the young than is generally assumed.

As it is well known, the war has caused the question of the frequency of carcinoma in soldiers (i.e., younger men) to be taken up again for discussion, and some investigators have observed carcinoma with astonishing frequency. As a control, Waetzold (12) has further summarized the number of carcinomatous individuals in the German army in ten years before the outbreak of the war, and in this period has found no less than 53 cases in men under thirty-five years (amongst which are 32 cases in soldiers of the age of nineteen to twenty-five years). Waetzold points out that the current opinion that carcinoma is a disease belonging to mature age (that is the years from forty to fifty) will no longer hold good.

Furthermore it must not be forgotten that some sorts of carcinoma develop with equal frequency in younger and in elder individuals, perhaps even with greater frequency in younger. Thus the common occurrence of Röntgen ray carcinoma in young men is a well established fact. Observations on the Bilharzia carcinoma, furthermore, indicate that this form of cancer also does not specially attack older individuals.

Hence, the power of the Spiroptera carcinoma to develop not only in old but also in young animals is a fact which is by no means without analogies among the tumors of man.

And for this reason Bullock and Rohdenburg's use of this fact as an argument against the classing of the Spiroptera carcinoma among the true neoplasms is quite unjustified.

But even if, in spite of all uncertainty, we might really venture to take it for granted that the majority of carcinomata in man and animals only begin their development in individuals that had reached an advanced age, we are not therefore justified in considering a more advanced age as, in the strict sense of the word, a truly predisposing factor in the development of such tumors. As is generally recognized, chronic irritation is a very important factor in the pathogenesis of numerous tumors. Their predominant occurrence in a developed state in older individuals need not, then, in itself mean anything but that most of the etiological

factors, in order to produce tumor at all, would have to act for such a long period that the tumors could not possibly appear except in individuals who for a great number of years had been exposed to the influence of the factor or factors in question and eo ipso must belong to older age-classes.

And the cause of the late appearance of the cancer might further, with regard to certain forms of cancer, be this, that only adult individuals were at all exposed to the etiological factors that produce these tumors, and that their occurrence would thus be postponed till a later age.

If, for example, we must assign a certain significance to the abuse of alcohol in the etiology of esophageal carcinoma, as generally assumed, the almost exclusive occurrence of this carcinoma in men past forty years of age could be accounted for simply by the fact that children are very rarely drunkards. And conversely, the frequent early occurrence of Bilharzial carcinoma might be a consequence of the fact that Bilharzial infection often takes place in childhood.

On the other hand, the frequent occurrence of Röntgen ray carcinoma in young men can hardly be explained analogically. Here the cause must rather be assigned to the fact that this irritant acts with greater intensity than the majority of irritants commonly in action, and is so vigorous in its effect that in a considerable number of individuals it is able to produce dermatitis and cancer of the skin even after a few years.

That Spiroptera carcinoma may occur in young animals might, perhaps, be accounted for in like manner.

From what has here been stated alone, it will appear with sufficient clearness that the generally accepted doctrine that "cancer age" must be taken as a proof that an advanced age in itself—as a special constitutional property of the organism—is a predisposing factor in the development of cancer, is in need of revision.

As a real proof of the existence of an age-predisposition in the strictest sense of the word, it would be required that older animals exposed for equally long periods to the same effective influence as young animals, more frequently become carcinomatous than the latter. But such a proof does not exist.

SUMMARY

By transmission of *Spiroptera neoplastica* (*Gongylonema neoplasticum*) to black and white rats and white mice, the development of neoplasms can be induced in the fundus of the stomach, and in rats in the tongue also. These neoplasms possess exactly the same histological structure as malignant epitheliomata (keratinizing squamous-cell carcinomata) in man and animals. They grow invasively into connective tissue and muscular tissue, and produce metastases in lymph-nodes, perineural lymphatic spaces, the lung, and the peritoneum. They continue their growth whether or not the *Spiroptera* (as observed in the tongue) disappear entirely or only partly. They are transplantable, and when transplanted grow invasively into organs and tissues. Neither the metastases nor the transplanted tumors contain *Spiroptera*, which have no share in their development and growth.

That these tumors are true carcinomata cannot, thus, be doubted, and the fact that they may occur in younger animals does not diminish our right to range them among the true malignant neoplasms.

REFERENCES.

- (1) FIBIGER, JOHANNES: Recherches sur un nématode et sur sa faculté de provoquer des néoformations papillomateuses et carcinomateuses dans l'estomac du Rat. Académie Royale des sciences et des lettres de Danemark. Extrait du Bulletin de l'année 1913, No. 1.
- (2) FIBIGER, JOHANNES: Untersuchungen über eine Nematode (*Spiroptera* sp. n.) und deren Fähigkeit, papillomatöse und carcinomatöse Geschwulstbildungen im Magen der Ratte hervorzurufen. Ztschr. f. Krebsforsch., 1913, xiii, 217.
- (3) FIBIGER, JOHANNES: Sur le développement de tumeurs papillomateuses et carcinomateuses dans l'estomac du rat sous l'action d'un ver nématode. Travaux de la troisième conférence internationale pour l'étude du cancer, 1913, Bruxelles, 1914.
- (4) FIBIGER, JOHANNES: Weitere Untersuchungen über das Spiropteraeacarcinom der Ratte. Ztschr. f. Krebsforsch., 1914, xiv, 295.
- (5) FIBIGER, JOHANNES AND DITLEVSEN H.: Contributions to the biology and morphology of spiroptera (*Gongylonema* *neoplastica* n. sp. Mindeskraft for Japetus Steenstrup, 1914.
- (6) FIBIGER, JOHANNES: Undersøgelser over Spiroptera neoplasticas Virkning paa Mus. 16'Skandinaviske Naturforskermøte i Kristiania 1916.

- (7) FIBIGER, JOHANNES: Ueber Disposition der Ratten und Mäuse für die Wirkungen der Spiroptera neoplastica. Centralbl. f. allg. Path. u. path. Anat., 1916, xxvii.
- (8) FIBIGER, JOHANNES: Investigations on the Spiroptera Cancer, III. On the transmission of Spiroptera neoplastica (*Gongylonema* n.) to the rat as a method of producing cancer experimentally. Det kongelige Danske Videnskabernes Selskab (Académie Royale des sciences et des lettres de Danemark). Biologiske Meddelelser I₉., 1918.
- (9) FIBIGER, JOHANNES: Investigations on the Spiroptera cancer, IV. Spiroptera cancer of the tongue in rats. Det kongelige Danske Videnskabernes Selskab. Biologiske Meddelelser I₁₀., 1918.
- (10) FIBIGER, JOHANNES: Investigations on the Spiroptera cancer, V. On the growth of small carcinomata and on predisposition to Spiroptera cancer in rats and mice. Det kongelige Danske Videnskabernes Selskab. Biologiske Meddelelser I₁₁., 1918.
- (11) FIBIGER, JOHANNES: Investigations on the Spiroptera cancer, VI. A transplantable Spiroptera carcinoma of the mouse. Det kongelige Danske Videnskabernes Selskab. Biologiske Meddelelser I₁₄., 1919.
- (12) WAETZOLD: Ztschr. f. Krebsforsch., 1919.

INDEX

Adaptation, virulence or.....	1
Age, cancer, some remarks on.....	367
Anaplasia, v. Hansemann's theory of, critical review of, in light of modern knowledge of genetics.....	181
Bercovitz, N., Cancer in Hainan, China. A preliminary statistical study of 131 operations with special reference to age incidence, anatomical distribution, and etiology.....	229
Blood, chemical composition of, in cancer.....	349
Bovie, W. I., Recent advances in our knowledge of physiological effects of radiation (summary).....	75
Bullock, F. D., and Rohdenburg, G. L., Experimental "carcinomata" of animals and their relation to true malignant tumors (summary).....	59
Cancer age, some remarks on.....	367
— among wage earners, mortality statistics of.....	235
—, chemical composition of blood in.....	349
— immunity, importance of lymphocyte in.....	57
— in Hainan, China. A preliminary statistical study of 131 operations with special reference to age incidence, anatomical distribution, and etiology.....	229
— — Norway, cost of.....	203
—, incidence of, in general population, observations on.....	235
—, mortality from, recent changes in.....	78
—, production of, somatic mutations as factor in.....	181
— rate in mice, effect of continued inbreeding on.....	65
— — mice of hybrid strains.....	65
Carcinoma and sarcoma cells, living, effects of x-ray irradiation on, in tissue cultures in vitro.....	95
—, mammary, in cat.....	19
"Carcinomata," experimental, of animals and their relation to true malignant tumors.....	59
Carcinomata, spiroptera, and their relation to true malignant tumors.....	367
Cat, carcinoma, mammary, in.....	19
Chemistry of blood in cancer.....	349
China, cancer in, statistical study of.....	229
Clowes, G. H. A., Preliminary note on action exerted by antagonistic electrolytes in electrical resistance of emulsion membranes (summary).....	86
—, Preliminary note on electrical resistance of tumor tissues (summary).....	86
Cost of cancer in Norway.....	203
Dental region, tumors of, lipoids in.....	263
Dewey, K. W., Lipoids in tumors of dental region.....	263
Duane, W., On measurement of x-ray dosage (summary).....	72

Dublin, L. I., Mortality statistics of cancer among wage earners: with observations on the comparative incidence of the disease in the general population.....	235
Electrolytes, antagonistic, action exerted by, on electrical resistance of emulsion membranes.....	86
Ewing, J., Some late and distant effects of radium (summary).....	45
Fibiger, J., On Spiroptera carcinomata and their relation to true malignant tumors; with some remarks on cancer age.....	367
Fischer, A., Multiple tumors of mouse mamma: Are they independent or metastatic?.....	325
Gade, F. G., Cost of cancer in Norway.....	203
Hainan, China, cancer in, statistical study of.....	229
v. Hansemann's theory of anaplasia, critical review of, in light of modern knowledge of genetics.....	181
Heat, effect of, upon tumor tissue.....	54
Hoffman, F. L., Recent changes in mortality from cancer (summary).....	78
Holmes, H. F., Slye, M., and Wells, H. G., Comparative pathology of tumors of the testis (summary).....	53
—, —, —, Primary spontaneous tumors of the testicle and seminal vesicle in mice and other animals. XII. Studies in the incidence and inheritability of spontaneous tumors in mice.....	207
Immunity, cancer, importance of lymphocyte in.....	57
Inbreeding, continued, effect of, on cancer rate in mice.....	65
Incidence and inheritability of spontaneous tumors of mice, XII. Primary spontaneous tumors of testicle and seminal vesicle in mice and other animals.....	207
Inheritability and incidence of spontaneous tumors in mice.....	207
Itami, S., Mammary carcinoma in cat.....	19
—, Investigation of power of mesodermal derivatives to immunize mice against transplantable tumors.....	23
Kimura, N. Effects of x-ray irradiation on living carcinoma and sarcoma cells in tissue cultures in vitro.....	95
Lathrop, A. E. C., and Loeb, L., Cancer rate in hybrid strains (summary).....	65
—, —, Effect of continued inbreeding on cancer rate in mice (summary).....	65
—, —, Further investigations on origin of tumors in mice. IV. The tumor incidence in later generations of strains with observed tumor rate.....	137
Lipoids in tumors of dental region.....	263
Loeb, L., and Lathrop, A. E. C., Cancer rate in hybrid strains (summary).....	65
—, —, Effect of continued inbreeding on cancer rate in mice (summary).....	65
—, —, Further investigations on origin of tumors in mice. IV. The tumor incidence in later generations of strains with observed tumor rate.....	137
Lymph-nodes in immune rats.....	44
Lymphocyte in cancer immunity, importance of.....	57
Mamma, mouse, tumors, multiple, of: Are they independent or metastatic?.....	325
Mann, F. C., Attempts to obtain transplantable tumor in higher species of animals.....	331

Membranes, emulsion, action exerted by antagonistic electrolytes on the electrical resistance.....	86
Mesodermal derivatives, power of, to immunize mice against transplantable tumors.....	23
Mice, cancer rate in, effect of continued inbreeding on.....	65
—, ——— hybrid strains.....	65
—, immune, size of spleen in.....	281
—, power of mesodermal derivatives to immunize, against transplantable tumors.....	23
—, tumor incidence in later generations of strains with observed tumor rate.....	137
—, tumors of, and spirochetes, relation between.....	89
—, ———, further investigations on origin of. IV. The tumor incidence in later generations of strains with observed tumor rate.....	137
—, —, primary spontaneous, of testicle and seminal vesicle in.....	207
—, —, spontaneous, of, incidence and inheritability of, XII. Primary spontaneous tumors of testicle and seminal vesicle in mice and other animals.....	207
Mortality from cancer, recent changes in.....	78
Mouse mamma, tumors, multiple, of: Are they independent or metastatic?.....	325
Mueller, T., Relation between spirochetes and mouse tumors (summary)....	89
Mutations, somatic, as factor in production of cancer. A critical review of v. Hansemann's theory of anaplasia in light of modern knowledge of genetics.....	181
Norway, cost of cancer in.....	203
Origin of tumors in mice, further investigations on. IV. The tumor incidence in later generations of strains with observed tumor rate.....	137
Prime, F., and Wood, F. C., Effect of x-ray on tumors (summary).....	49
Proceedings of the American Association for Cancer Research, 11th Annual Meeting.....	43
Lymph-nodes in immune rats.....	44
Some late and distant effects of radium.....	45
Effect of x-ray on tumors.....	49
Comparative pathology of tumors of testis.....	53
Effect of heat upon tumor tissue.....	54
Further studies on importance of lymphocyte in cancer immunity.....	57
Experimental "carcinomata" of animals and their relation to true malignant tumors.....	59
Effect of continued inbreeding on cancer rate in mice.....	65
Cancer rate in hybrid strains.....	65
Quantitative study of physiological action of radium.....	69
Measurement of x-ray dosage.....	72
Recent advances in our knowledge of the physiological effects of radiation.....	75
Recent changes in mortality from cancer.....	78
Preliminary note on action exerted by antagonistic electrolytes on electrical resistance of emulsion membranes.....	86
Preliminary note on electrical resistance of tumor tissues.....	86
Relation between spirochetes and mouse tumors.....	89

Radiation, physiological effects of.....	75
Radium, physiological action of, quantitative study of.....	69
—, some late and distant effects of.....	45
Rats, immune, lymph-nodes in.....	44
Redfield, A., Quantitative study of physiological action of radium (summary).....	69
Resistance, electrical, of emulsion membranes, action exerted by antagonistic electrolytes on.....	86
—, —, — tumor tissues.....	86
Rohdenburg, G. L., and Bullock, F. D., Experimental "carcinomata" of animals and their relation to true malignant tumors (summary).....	59
Sarcoma and carcinoma cells, living, effects of x-ray irradiation on, in tissue cultures in vitro.....	95
Seminal vesicle and testicle, tumors, primary spontaneous, of, in mice and other animals.....	207
Sittenfield, M. J., Further studies on importance of lymphocyte in cancer immunity (summary).....	57
Slye, M., Holmes, H. F., and Wells, H. G., Comparative pathology of tumors of the testis (summary).....	53
—, —, —, Primary spontaneous tumors of the testicle and seminal vesicle in mice and other animals. XII. Studies in the incidence and inheritability of spontaneous tumors in mice.....	207
Somatic mutations as factor in production of cancer. A critical review of v. Hansemann's theory of anaplasia in light of modern knowledge of genetics.....	181
Spirochetes and mouse tumors, relation between.....	89
Spiroptera carcinomata and their relation to true malignant tumors.....	367
Spleen, size of, in mice, immune.....	281
Statistics, mortality, of cancer among wage earners.....	235
Stevenson, H. N., Effect of heat upon tumor tissue (summary).....	54
Stone, W. S., and Theis, R. C., Study of chemical composition of blood in cancer.....	349
Testicle and seminal vesicle, tumors, primary spontaneous, of, in mice and other animals.....	207
Testis, tumors of, comparative pathology of.....	53
Theis, R. C., and Stone, W. S., Study of chemical composition of blood in cancer.....	349
Tissue cultures in vitro, effects of x-ray irradiation on living carcinoma and sarcoma cells in.....	95
Tumor incidence in later generations of strains with observed tumor rate..	137
— tissue, effect of heat upon.....	54
— —, electrical resistance of.....	86
Tumors, effect of x-ray on.....	49
— in dental region, lipoids in.....	263
—, malignant, relation of Spiroptera carcinomata to.....	367
—, mouse, and spirochetes, relation between.....	89

Tumors, mouse, further investigations on origin of. IV. The tumor incidence in later generations of strains with observed tumor rate.....	137
—, multiple, of mouse mamma: Are they independent or metastatic?....	325
— of testis, comparative pathology of.....	53
—, primary spontaneous, of testicle and seminal vesicle in mice and other animals.....	207
—, transplantable, attempts to obtain, in higher species of animals . . .	331
—, —, power of mesodermal derivatives to immunize mice against . . .	23
Virulence or adaptation?.....	1
Wage earners, cancer among, mortality statistics of.....	235
Wells, H. G., Slye, M., and Holmes, H. F., Comparative pathology of tumors of the testis (summary).....	53
—, —, —, Primary spontaneous tumors of the testicle and seminal vesicle in mice and other animals. XII. Studies in the incidence and inheritability of spontaneous tumors in mice	207
Whitman, R. C., Somatic mutations as factor in production of cancer. A critical review of v. Hansemann's theory of anaplasia in light of modern knowledge of genetics.....	181
Woglom, W. H., Lymph-nodes in immune rats (summary)....	44
—, Size of spleen in immune mice.	281
—, Virulence or adaptation?.....	1
Wood, F. C., and Prime, F., Effect of x-ray on tumors (summary)	49
X-ray dosage, measurement of.....	72
—, effect of, on tumors.....	49
— — irradiation, effects of, on living carcinoma and sarcoma cells in tissue cultures in vitro	95

RC
261
A24
v.3-4

The American journal of
cancer

Biological
& Medical
Serials

PLEASE DO NOT REMOVE
CARDS OR SLIPS FROM THIS POCKET

UNIVERSITY OF TORONTO LIBRARY

STORAGE

